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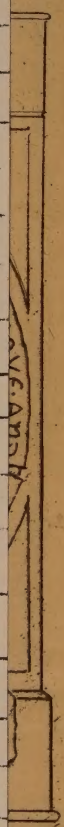

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BY

EDWARD C. KENDALL, M.S., PH.D., D.SC.

THE MAYO FOUNDATION, ROCHESTER, MINNESOTA.

American Chemical Society
Monograph Series

BOOK DEPARTMENT

The CHEMICAL CATALOG COMPANY, *Inc.*

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GENERAL INTRODUCTION

American Chemical Society Series of Scientific and Technologic Monographs

By arrangement with the Interallied Conference of Pure and Applied Chemistry, which met in London and Brussels in July 1919, the American Chemical Society was to undertake the production and publication of Scientific and Technologic Monographs on chemical subjects. At the same time it was agreed that the National Research Council, in coöperation with the American Chemical Society and the American Physical Society, should undertake the production and publication of Critical Tables of Chemical and Physical Constants. The American Chemical Society and the National Research Council mutually agreed to care for these two fields of chemical development. The American Chemical Society named as Trustees, to make the necessary arrangements for the publication of the monographs, Charles L. Parsons, Secretary of the American Chemical Society, Washington, D. C.; John E. Teeple, Treasurer of the American Chemical Society, New York City; and Professor Gellert Alleman of Swarthmore College. The Trustees have arranged for the publication of the American Chemical Society series of (a) Scientific and (b) Technologic Monographs by the Chemical Catalog Company of New York City.

The Council, acting through the Committee on National Policy of the American Chemical Society, appointed the editors, named at the close of this introduction, to have charge of securing authors, and of considering critically the manuscripts prepared. The editors of each series will endeavor to select topics which are of current interest and authors who are recognized as authorities in their respective fields. The list of monographs thus far secured appears in the publisher's own announcement elsewhere in this volume.

The development of knowledge in all branches of science, and especially in chemistry, has been so rapid during the last fifty years and the fields covered by this development have been so varied that it is difficult for any individual to keep in touch with the progress in branches of science outside his own specialty. In spite of the facilities for the examination of the literature given by Chemical Abstracts and such compendia as Beilstein's Handbuch der Organischen Chemie, Richter's Lexikon, Ostwald's Lehrbuch der Allgemeinen Chemie, Abegg's and Gmelin-Kraut's Handbuch der Anorganischen Chemie and the English and French Dictionaries of Chemistry, it often takes a great deal of time to coördinate the knowledge available upon a single topic. Consequently when men who have spent years in the study of important subjects are willing to coördinate their knowledge and present it in concise, readable form, they perform a service of the highest value to their fellow chemists.

It was with a clear recognition of the usefulness of reviews of this character that a Committee of the American Chemical Society recommended the publication of the two series of monographs under the auspices of the Society.

Two rather distinct purposes are to be served by these monographs. The first purpose, whose fulfilment will probably render to chemists in general the most important service, is to present the knowledge available upon the chosen topic in a readable form, intelligible to those whose activities may be along a wholly different line. Many chemists fail to realize how closely their investigations may be connected with other work which on the surface appears far afield from their own. These monographs will enable such men to form closer contact with the work of chemists in other lines of research. The second purpose is to promote research in the branch of science covered by the monograph, by furnishing a well digested survey of the progress already made in that field and by pointing out directions in which investigation needs to be extended. To facilitate the attainment of this purpose, it is intended to include extended references to the literature, which will enable anyone interested to follow up the subject in more detail. If the literature is so voluminous that a complete bibliography is impracticable, a critical selection will be made of those papers which are most important.

The publication of these books marks a distinct departure in the policy of the American Chemical Society inasmuch as it is a serious attempt to found an American chemical literature without primary regard to commercial considerations. The success of the venture will depend in large part upon the measure of coöperation which can be secured in the preparation of books dealing adequately with topics of general interest; it is earnestly hoped, therefore, that every member of the various organizations in the chemical and allied industries will recognize the importance of the enterprise and take sufficient interest to justify it.

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Preface

Investigations concerning the thyroid gland have been carried out in turn by clinicians, surgeons, pathologists and chemists. In the many books on the subject the chemical investigations of the thyroid gland have been well covered in a few of the introductory pages, and the remainder of the book has been devoted to other phases of the problem. In 1919 the Board of Editors of the American Chemical Society Monographs decided that the results of the chemical studies should be presented in monograph form. I was reluctant to prepare my manuscript until after the chemical nature of thyroxine had been definitely established. Because of the reasons which are briefly outlined, the chemical investigation of thyroxine was prolonged and this monograph which was among the first to be advertised now assumes forty-seventh place in the series. Much of the work which is presented, however, has been completed within the last two or three years and any monograph on the chemistry of the thyroid prepared before the present year would have been out of date almost before the printing had been completed.

What has been assembled in the monograph has been chosen from the viewpoint of a chemist and with a desire to correlate the activity of the thyroid gland with reactions which involve oxidation. It is fully realized that the complex mechanism through which the activity of the thyroid gland is made manifest is not understood; on the other hand, the clinical and physiologic results are clear and quantitative. This is not only an invitation but a challenge to the chemist. During the last few years many of the hypotheses which have been suggested in regard to the function of the thyroid have been shown to be inadequate and those that remain are confined within narrow limits. In a corresponding degree, the number of possible experimental approaches to the problem have been increased and definite steps have been taken within the field of physical chemistry. Neither the clinical nor the physiological investigation of the thyroid gland is now empirical.

The chemical problems associated with the thyroid gland are fascinating; thyroxine bridges the gap between living protoplasm and the expression of what may be called personality or temperament. Thyroxine

has a definite place in the study of normal and abnormal mental processes. Whether or not it functions as an enzyme or influences the velocity of enzyme action may be subject to controversy, but the processes which it does affect can be shown to be of fundamental importance not only to the physiologic chemist but to the social and economic activities of man.

The theoretical deductions in this monograph have been largely based on three contributions: the clinical classification of goiter and the use of iodine in exophthalmic goiter which have been suggested by Plummer; the isolation of thyroxine from the gland; and the extensive studies in basal metabolism and nitrogen metabolism in relation to thyroxine and thyroid diseases which have been carried out by Boothby and his coworkers. These three related subjects are a part of the investigation of the activity of the thyroid gland which was initiated and carried out in The Mayo Clinic largely through the work of Plummer. The important contributions of C. H. Mayo and his associates in the surgery of goiter and of Wilson, McCarty, Broders and their associates on the pathologic investigations of goiter, on account of the limited scope of the monograph, can only be referred to briefly.

The theory that an abnormal product is secreted by the thyroid gland is presented with full knowledge that no other gland has been shown to produce a qualitatively as well as a quantitatively altered secretion. It is believed that the clinical evidence taken together with the definite knowledge of the physiologic activity of thyroxine furnishes data which can best be explained by the theory presented by Plummer.

The experimental results to which reference has been made have been limited to those that establish the physiologic activity of thyroxine and those necessary to relate the action of the thyroid gland to the normal physiology of the human being. Reference numbers have been placed after the author's name only in those cases where the bibliography lists more than one article by the author. The monograph must be regarded as a photograph of an ever-changing scene: some small object recorded but not conspicuous may appear subsequently in a position of prominence. There are many questions left unanswered: time will bring many additions and possibly many alterations.

E. C. KENDALL.

Rochester, Minnesota.
July 1, 1928.

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Chapter I

Early Chemical Investigations of the Thyroid

Progress in biological research does not result in simplification of the problem investigated; rather it leads but to further study of more intricate relationships than were at first apparent. This is well illustrated by the facts which have slowly been accumulated concerning the chemistry of the thyroid gland.

The condition associated with lack of thyroid activity was recognized in the early eighties of the last century and the effect of the removal of the thyroid was demonstrated by Kocher,[284] in 1883. The prevalence of goiter in Europe had prompted the clinical investigation of thyroid disturbances and in 1883 a commission,[412] was appointed to investigate the problem of myxedema.

It was not until relief from hypothyroidism was brought about by the clinician that chemists became actively interested in an investigation of the gland. Murray, in England, in 1891 first used an extract of the thyroid gland to treat a patient who was suffering from hypothyroidism. The following year Fox and Mackenzie obtained similar results, and in 1893, Ord and White administered twenty drops, daily, of a glycerine extract of sheep thyroid in a case of myxedema. They drew the following conclusions: (1) The urine is increased in volume; (2) the nitrogen excreted in the urine exceeds the total quantity of the nitrogen in the food; (3) elimination of phosphoric acid and chlorine is practically unaffected; (4) the increased nitrogen excreted is chiefly in the form of urea; (5) the body weight is rapidly diminished, and (6) the temperature of the body is raised.

In Germany, the work of Leichtenstern [307, 308] and Wendelstadt, Emminghaus and Reinhold, showed that the condition of hypothyroidism could be relieved. Wendelstadt, in 1893, treated twenty-two cases of hypothyroidism with fresh sheep's thyroid and desiccated thyroid.

Mendel, Vermehren, Napier, Dennig,[122, 123] Bleibtreu and Wendelstadt, and Roos,[434] also showed that thyroid gland not only relieved the

condition of hypothyroidism but also brought about loss of weight and increased protein breakdown.

Roos,[430] working in Baumann's laboratory, reported an investigation in 1895 concerning the physiological activity of the thyroid gland and showed that administration of thyroid caused an increase in the nitrogen output.

This work was completed just before Baumann published the important discovery that iodine was a normal constituent of the gland. This fact was announced by Baumann in December, 1895, and it was soon followed by an extended investigation by this investigator and others concerning the presence of iodine in the thyroid gland and the chemical nature of the organic compound to which the iodine was bound. Although Kocher [285] suggested in 1895 that the relation of iodine to the physiological activity of the thyroid gland should be investigated, Baumann was apparently unacquainted with this suggestion, and he stated that the immediate cause of his investigation of the chemical nature of the thyroid gland was a suggestion of Kraske, which was based on the clinical results of Emminghaus and Reinhold. That Baumann did not anticipate the presence of iodine in the thyroid is clearly shown by the fact that he was surprised when he saw the iodine liberated with nitric acid from an alkaline fusion of a product separated from the gland. He said,[32] "When I first made this observation, I believed anything else rather than that the iodine belonged to my substance,"—iodothyrin.

Baumann showed that thyroid material could be boiled with 10 per cent sulfuric acid and a fraction could be separated which was from 0.2 to 0.5 per cent of the dry weight of the gland. This fraction of the thyroid possessed the typical physiological activity of the gland and it contained about 10 per cent of iodine. It was named thyreo-iodin and later iodothyrin. When administered to patients suffering either from hypothyroidism or from goiter, it brought about the same improvements which were produced by desiccated thyroid, and for this reason iodothyrin was further extensively investigated for some clue to its chemical nature.

The effect which Baumann's discovery of iodine in the thyroid gland had on biochemists and clinicians was similar to the announcement of the discovery of insulin. Many papers were published. The crystals of pure iodine separated from the thyroid gland were demonstrated at society meetings and high hopes were entertained of the prompt solution

of problems concerning the action of iodine and iodine compounds in the animal organism.

Drechsel,[133, 134] Hofmeister,[230] Oswald,[379] and Roos [433] investigated the occurrence of other iodine compounds in nature and determined the influence of iodized proteins to see whether the iodine compound found in the thyroid could be prepared by combining iodine with protein in the laboratory. They showed that proteins so treated did not possess the characteristic physiological action of iodothyrin. Oswald showed that all the iodine in protein after treatment with iodine was not present as di-iodotyrosine, although a large proportion of the iodine apparently was attached to the tyrosine molecule.

Although Baumann's discovery stimulated chemical investigation of the thyroid, his was not the first work concerned with an attempt to identify the constituents of the gland. Bubnow, in 1883, and Gourlay, in 1894, had investigated the proteins of the gland. Roos [430] carried out a similar investigation. The work was confined to an attempt to fractionate the proteins. Investigations having the same objective were reported by Morkotun, Drechsel,[133, 134] Blumenthal, Tambach, Hutchison,[245, 246] and others, but the proteins of the thyroid were studied in greatest detail by Oswald.[376]

Oswald dissolved fresh-ground thyroid glands in physiological salt solution and added an equal volume of saturated ammonium sulfate. The precipitate was redissolved in water and again precipitated with ammonium sulfate. The snow-white precipitate was dialyzed until free from sulfate, and was then precipitated with 95 per cent alcohol. The same fraction could also be obtained by dissolving the protein in dilute sodium hydroxide and precipitating with acetic acid. This iodine-containing protein was called iodothyreoglobulin and it was soon shown that this substance also produced the clinical improvement in cases of thyroid deficiency as desiccated thyroid did.

Hutchison [245, 246] showed that there were two proteins, a nucleo albumin and colloid matter. The nucleo albumin was similar to that found in other organs. The colloid matter which was present in large amounts in the thyroid was contained in the acini and was apparently identical with Oswald's thyreoglobulin. Hutchison [246] analyzed many samples of the fractionated proteins for their iodine content and found it to vary between 0.12 and 0.462 per cent of iodine. Oswald [376] gave the analysis of iodothyreoglobulin in per cent as follows: Carbon 52.21, hydrogen 6.83, nitrogen 16.59, iodine 1.66, sulfur 1.86. The iodine content of iodothyreoglobulin was shown to be not constant, and separation

of the material did not materially increase the percentage of the iodine content above that of desiccated thyroid. When iodothyreoglobulin was treated with 10 per cent sulfuric acid it was decomposed and iodothyrin could be separated. By the separation of thyreoglobulin, before treatment with sulfuric acid, it appeared to be possible to increase the iodine content of the iodothyrin, and one preparation was secured which contained 14.48 per cent of iodine.

The physiological action of the two products, iodothyrin and iodothyreoglobulin, was investigated in cases of goiter, obesity and in experimental hypothyroidism. Experiments were also made to determine the influence of these compounds on the nervous system. From this work it was concluded that thyreoglobulin contained the full equivalent of the essential constituent of the thyroid gland; while iodothyrin produced similar effects some investigators became convinced that iodothyrin was not the actual active agent of the thyroid gland, but was a decomposition product of the essential constituent.

In addition to iodothyrin and iodothyreoglobulin another product was prepared by Fränkel,[161, 162] who believed it to contain the active agent of the thyroid gland. This preparation, thyreo-antitoxin, was made by precipitating the proteins of the thyroid gland with acetic acid and lead acetate. The residue in the filtrate was dissolved in alcohol and precipitated with acetone or ether. When redissolved in water it did not precipitate with lead, but did precipitate with phosphotungstic acid and similar reagents. Thyreo-antitoxin has not been used extensively as it was soon shown to possess little or none of the typical activity of the thyroid gland.

CHEMICAL NATURE OF IODOTHYRIN AND IODOTHYREOGLOBULIN

Baumann [32] prepared iodothyrin by hydrolyzing the proteins of the thyroid for several hours with boiling 10 per cent sulfuric acid. The solution was cooled and filtered and the residue extracted with boiling 85 per cent alcohol. Evaporation of the alcohol left a brown, amorphous material which was freed from fat and fatty acid with petroleum ether. The material was then dissolved in 1 per cent sodium hydroxide and reprecipitated with sulfuric acid.

It was shown that this material did not give characteristic protein reactions. Nürnberg [371] found that after iodothyrin was autoclaved for six hours at six atmospheres the solution gave a Millon's reaction and that if iodothyrin was reduced in alcohol with sodium the solution gave a positive tryptophane reaction. He concluded that iodine was attached to

either tyrosine or tryptophane, and that so far as *para*dimethaminobenzaldehyde is specific for tryptophane, so far was it probable that iodothyrin contained an iodo derivative of tryptophane. These two amino acids therefore were associated with the possible structure of the thyroid hormone within the first few years after the discovery of iodine by Baumann, although which of these amino acids was the carrier of the iodine was not determined until eighteen years later.

Iodothyrin was compared with other naturally occurring iodine compounds, one of the most interesting of which was iodogorgoic acid. This was separated by Drechsel [134] and was first described as iodo-amino butyric acid. Henze [213, 214] and Wheeler and Jamieson showed that the correct structure of this compound was 3,5-di-iodotyrosine.

Through physiological tests it was shown that iodothyrin could not be di-iodotyrosine since the latter compound did not simulate desiccated thyroid in physiological activity (Strouse and Voegtlin).

Nürnberg [372] also investigated iodothyreoglobulin and attempted to separate the iodine-containing compound after hydrolysis of the protein with barium hydroxide. The material was heated with saturated barium hydroxide for thirty hours, and was then filtered from the insoluble material and the barium hydroxide which crystallized from solution. The filtrate was treated with carbon dioxide and dilute sulfuric acid to remove the barium. This filtrate did not give the biuret reaction and did not contain inorganic iodine. From this filtrate he was not able to isolate any di-iodotyrosine.

It is interesting to note that in the method used by Nürnberg, the barium-insoluble products of the hydrolyzed thyreoglobulin were filtered out and were not examined; he attempted to isolate the iodine compound from the barium-hydroxide soluble portion only. Had he investigated the products insoluble in barium hydroxide in respect to their physiological behavior it is possible that the isolation of the iodine compound might have been accomplished several years previous to the date of its final separation in 1914.

Oswald [380] also carried on experiments concerning the hydrolysis of iodothyreoglobulin. He was impressed with the instability of the iodine-containing compound and therefore used mild hydrolytic agents, and treated the proteins with cold barium hydroxide. Since his solutions were not heated he did not obtain a barium-insoluble fraction similar to Nürnberg's and he did not isolate any definite iodine compound.

Working with digestive enzymes he showed that trypsin broke off about 75 per cent of the iodine. When iodothyreoglobulin was digested

for ten months and then treated with an extract of erepsin for three months, the solution contained crystals of tyrosine and 91 per cent of the iodine was present as iodide. The solution did not produce any effect on the excretion of nitrogen, and Oswald concluded that all methods of hydrolytic splitting of iodothyreoglobulin (heating with acid or alkali, digesting with trypsin and erepsin) result in breaking iodine off the protein.

It is to be noted in respect to the stability of the iodine that Oswald continued the treatment with the enzyme for a long time. Pick and Pineles, and later Cameron and Carmichael,[94] showed that, if the thyroid proteins are treated for a short time only, the iodine is not broken off and the hydrolyzed products possess the same physiological activity as desiccated thyroid.

Roos [432] digested thyroid material with pepsin and extracted the products with alcohol. There was no effective separation of the physiologically active material by this treatment. He furthermore showed that the residue after the extraction of the proteins of the gland with physiological salt did not possess physiological activity. In order to show the stability to alkali Roos dissolved iodothyrin in 2 per cent potassium hydroxide and reprecipitated it with an acid. Iodothyrin did not lose its physiological activity when treated in this manner. The criterion which he used was the effect of iodothyrin on the clinical symptoms of two patients with goiter.

Roos also boiled iodothyrin in 5 per cent potassium hydroxide, acidified the solution, dried the precipitate and mixed it with milk sugar. This was ground in a mortar and extracted with ether. When given to a patient with goiter in 1 gram doses, it failed to show the characteristic physiological effect. After this material had been tried on six patients he concluded that thyroid substance after boiling with potassium hydroxide was much less effective than the same weight of gland boiled with acid. It would thus appear that Roos,[432] in 1896, showed the important difference between acid and alkaline hydrolyzing agents.

Blum [49] determined the percentage of iodine in four samples of iodothyrin to be as follows: 4.5, 0.8, 0.92, 1.6. The iodine content appeared to depend upon the sample of gland used.

Through the work of Oswald,[376] Baumann,[34] Tambach, Hutchison,[245, 246] von Cyon and Oswald,[119] Baumann and Roos,[37] and others, it was shown that the iodine-containing compound of the thyroid does not exist free in the thyroid secretion, but is bound to the protein molecule. It therefore became evident that before it could be separated

in pure, crystalline form it would be necessary to find some method to hydrolyze it from the proteins to which it was firmly attached.

For about twenty years after the first investigation of thyreoglobulin and iodothyrin a successful fractionation of these products was not reported and our knowledge of the chemical constituents of the thyroid gland rested in this form. During this time, however, some unpublished investigations were carried on with the object of establishing the chemical nature of the iodine-containing compound. Thyroid material was fractionated in the laboratory of Müller,[359] until a product containing 30 per cent of iodine was separated.

In 1909, Pick and Pineles reviewed the problem of the relation between iodothyrin, iodothyreoglobulin and desiccated thyroid. Baumann and his collaborators, Goldmann and Roos, believed that iodothyrin contained the active constituent of the thyroid, and stated that the acute symptoms of tetany following the removal of the thyroid gland in dogs or cats was relieved by administration of iodothyrin. Pick and Pineles pointed out that the claim of Baumann that iodothyrin is the active constituent of the thyroid gland was supported by the work of Hildebrandt,[225] Spoto, Hofmeister,[229] and Irsai; however, Baumann and Goldmann,[36] Stabel, Gottlieb, and Notkin,[370] could not observe any relief of the tetany and cramps following removal of the thyroid. This difference in results was satisfactorily explained after the rediscovery of the parathyroid glands by Gley and by Kohn, and by proof that the tetany following removal of the thyroid gland was actually due to parathyroid deficiency and not to loss of thyroid function.

Pick and Pineles mention the clinical observations of Leichtenstern,[34] Ewald, Grawitz, and Hennig, who had investigated the activity of iodothyrin through its clinical effect. A more quantitative measure of the influence of iodothyrin was made by Magnus-Levy,[331] who compared the action of this substance with that of desiccated thyroid, using as a criterion the output of nitrogen and the effect on the basal metabolic rate. Magnus-Levy concluded that iodothyrin is identical with thyroid substance in its action although its activity is not proportional to its iodine content.

Pick and Pineles prepared a series of thyrodectomized goats and investigated the relief of hypothyroidism by the administration of desiccated thyroid, iodothyrin and products obtained by digesting the proteins of the thyroid gland with pepsin and trypsin. They found that the condition of hypothyroidism was relieved by the administration of desiccated thyroid and iodothyreoglobulin. With iodothyrin and the products

of long-continued enzyme hydrolysis they did not obtain any effect, and they concluded that all the split products of the thyroid gland are inert and cannot contain the active agent of this gland. When the gland was digested for a short time with pepsin some activity was present in the split products.

Pick and Pineles suggested that the effect of iodothyrin on the basal metabolism which had been shown by Magnus-Levy was brought about not by the iodothyrin administered but by the increased activity of the thyroid gland of the patient.

The results of Pick and Pineles, Isaac and von den Velden, von Fürth and Schwarz,[167] Oswald,[380] and Roos,[432] and others were evidence that hydrolysis of the iodine-containing compound from the proteins of the thyroid resulted in its decomposition.

Koch determined the physiological activity of the various fractions resulting from acid hydrolysis of the thyroid gland by means of the Hunt test, with acetonitril. The iodothyreoglobulin separated from the gland manifested thyroid activity equivalent to that of the original thyroid tissue, and the metaprotein fraction also possessed apparently equal activity. This last-mentioned fraction was only a small part of the total, and all the other products of hydrolysis were markedly less active. The amino acid fraction, after acid hydrolysis, still contained small amounts of iodine, but iodine in this form of combination was either extremely low or quite wanting in thyroid activity. The iodothyrin exhibited activity equivalent to about 75 per cent of that of desiccated thyroid but this fraction also represented only a small part of the total iodine.

The inactivity of the products of hydrolysis obtained by many investigators is in strong contrast to the effect of iodothyrin on the basal metabolic rate reported by Magnus-Levy (which was not made less significant by the explanation given by Pick and Pineles) and the marked clinical effect in some cases brought about by this substance. These results indicated that under certain conditions the iodine-containing compound is stable enough to withstand hydrolysis from the proteins of the thyroid.

SUMMARY

An iodine-containing compound is a constituent of the thyroid proteins. Acid hydrolysis results in the separation of a small fraction, iodothyrin, which contains iodine and possesses physiological activity similar to that of desiccated thyroid. The iodine compound has unique physiological properties and its effect cannot be produced with iodized

proteins or by the naturally occurring di-iodotyrosine. Early investigations indicated that if iodothyreoglobulin, the protein which contains the iodine compound, is hydrolyzed with enzymes, with acid, or with alkali, its essential physiological activity was destroyed. Sufficient positive results, however, were obtained to indicate that under some conditions the iodine-containing compound could be hydrolyzed from the protein without destruction of its physiological activity.

Chapter 2

The Isolation and Identification of Thyroxine

HYDROLYSIS OF THE THYROID PROTEINS WITH ALKALI

In order to determine the stability and diffusion of the iodine-containing compound after alkaline hydrolysis the author determined the percentage of the total iodine which would pass through a collodion sac after desiccated thyroid had been dissolved in various strengths of sodium hydroxide. It was shown that almost none of the iodine compounds passed through: this indicated the necessity for further hydrolysis.

Because acid hydrolysis of the thyroid material did not produce a satisfactory separation of the constituents, I investigated the effect of alkaline hydrolysis on the iodine-containing compound. It was shown that after thyroid material had been boiled for a few hours with 5 per cent sodium hydroxide, acidification of the solution precipitated approximately 40 per cent of the total iodine in a form insoluble in acids.

The acid-soluble portion administered to a normal dog or to normal adult human beings and to thyroid-deficient children failed to produce any physiological change which was similar to the effect of desiccated thyroid, and this fraction could be given in large amounts without the production of any of the results recognized as due to an excess of thyroid substance.

Although Roos,[432] in 1896, had studied the effect of alkali on the thyroid and, although Oswald and Nürnberg had subsequently hydrolyzed the protein with barium hydroxide, a quantitative investigation of the physiological effect of the acid-insoluble fraction had not been carried out by these investigators. It seemed essential to me to determine the physiological activity of this fraction. The products insoluble in acid after alkaline hydrolysis were administered to patients with myxedema, and it was shown that all of the signs and symptoms of hypothyroidism could be relieved.

It was then shown that this material had a marked effect on experimental animals. A group of six goats were given this material by subcutaneous injection; the total dose was approximately 50 mg. a day. One

goat of this group died on the eleventh day and the other five goats died within the following ten days. All the goats lost weight markedly and the electrocardiogram showed that the heart was apparently laboring under the influence of toxic products.

In thyroid-deficient children beneficial effects were observed which were entirely comparable with those of desiccated thyroid; adult human beings manifested toxic symptoms similar to those induced by desiccated thyroid, after the administration of a few milligrams a day of this material. A monkey died after 72 daily injections of a few milligrams of the acid-insoluble products, which contained 1.5 mg. of iodine. See Figures 23 and 24 on page 80.

The physiological activity of the acid-insoluble compounds which had withstood sodium hydroxide hydrolysis was encouraging evidence that the first step in breaking down thyroid material could be successfully carried out without complete destruction of the essential physiological activity, and it was hoped that the final isolation of the active agent in crystalline form could be accomplished without subjecting the iodine compound to any chemical treatment more drastic than the initial hydrolysis with sodium hydroxide.

Thyroid material in large amount was hydrolyzed with alkali, first with 2 per cent sodium hydroxide in alcohol and subsequently with 5 per cent sodium hydroxide in water. The resulting material was fractionated into the acid-soluble and acid-insoluble portions and the acid-insoluble or "A" fraction was actively investigated in an attempt to find some method by which the iodine-containing compound could be separated in pure form.

An organic solvent was not found which would separate the material from the black, tarry products with which it was associated. The ease and accuracy with which the iodine content of the various fractions could be determined furnished a reliable index of the value of any particular method of separation which was being investigated.

The iodine-containing compounds were not completely dialyzable. Successive solution of the material in alkali and precipitation with acid did not materially increase the iodine content. No heavy metal could bring about differential precipitation of the material, either in water or alcohol, so that it was felt that the compound either still existed in combination with another substance or else the complex, colloidal nature of the impurities was such that further drastic treatment was necessary in order to permit the isolation of the iodine-containing compound.

With this object in mind continued hydrolysis with barium hydroxide was tried and it was found that, after further heating of the acid-

insoluble products with 10 per cent barium hydroxide for twenty-four hours, some type of hydrolysis occurred and the precipitate obtained by acidification of the barium hydroxide solution was notably different from the material before treatment with this alkali.

However, the iodine-containing compounds were not found entirely in the barium hydroxide soluble fraction. About half of the total iodine was soluble in barium hydroxide and the remainder was insoluble. The insoluble portion was decomposed with sodium hydroxide and sodium sulfate and then both groups of hydrolyzed products were precipitated with acid. The iodine content of the dried acid-insoluble material had increased in both fractions.

With successive treatment of this kind the iodine content of the acid-insoluble portion was slowly increased and products containing 30, 35, 42, and finally 47 per cent of iodine were separated. This material was almost colorless and was easily soluble in alcohol and sodium carbonate, and it appeared probable that some other form of treatment might effect crystallization of the iodine-containing compound. The fraction which contained 47 per cent of iodine was therefore dissolved in a small amount of 95 per cent alcohol which was evaporated to a small volume on the water bath. Unintentionally the alcohol was entirely evaporated and when more alcohol was added to the dried residue a white crust of material which was insoluble in alcohol was left on the bottom of the container.

This was filtered off and was to have been discarded as probably physiologically inert. It was found, however, to contain iodine in organic combination which amounted to 60 per cent of the weight of the material. This small amount of the desiccated thyroid which did not weigh more than 50 mg. was soon shown to be soluble in alcohol containing sodium hydroxide or mineral acid, but insoluble in alcohol containing acetic acid. Solution of the material in alkaline alcohol followed by precipitation with acetic acid caused the compound to crystallize in sheaves of needles. This compound was first seen in crystalline form on December 25, 1914. (Fig. 1.)

At that time there was on hand a small amount of acid-insoluble products of alkaline hydrolysis which were further treated in order to separate in pure, crystalline form as much as possible of the iodine-containing compound. Approximately 200 mg. was secured.

Subcutaneous injection of this material into dogs produced the same typical changes which had been observed with the entire fraction of the acid-insoluble material and the administration of the compound in 0.5

mg. daily portions to a cretin and to a myxedematous patient was promptly followed by relief of all signs of thyroid deficiency.

It immediately became desirable to isolate this material on a large scale, and for this purpose glass flasks were discarded and a large galvanized iron tank was used to effect the alkaline hydrolysis. More of

FIG. 1.—Crystals of thyroxine as it separates from an alcoholic solution of its sodium salt after acidification with acetic acid.

the crystals were not isolated for many months, and it seemed probable at that time that the failure to isolate the material was due to the destruction of the iodine-containing compound by the effect of the galvanized iron. This no doubt was one of the reasons but another important one was the fact that the glands collected during the winter months contained but a small amount of iodine.

In place of the galvanized iron tank, large enameled kettles were used and many hundreds of pounds of thyroid material were treated with sodium hydroxide. In August, 1915, a small amount of the crystalline iodine compound was again isolated, but it was not until February, 1916, that a treatment of thyroid material which would consistently bring about a separation of the iodine compound was finally found. A method for the treatment of desiccated thyroid was eventually evolved by which

a small amount of the material could be separated from some samples of desiccated thyroid.

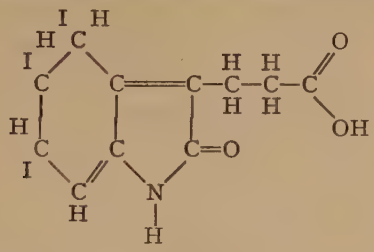
It was then decided to use fresh thyroid glands instead of desiccated material and for this treatment the enameled kettles were replaced with pure nickel containers holding approximately 35 liters. The fresh thyroid glands were hydrolyzed with 5 per cent sodium hydroxide; the sodium soaps of fatty acids were separated as an insoluble material by the addition of salt to the alkaline solution and the sodium hydroxide was neutralized with sulfuric acid. The resulting brown, flocculent precipitate was then filtered off and treated with barium hydroxide, and the iodine compound was finally crystallized from alkaline alcohol by acidification with acetic acid.

By the spring of 1917, 7 grams of the iodine-containing compound had been separated in pure, crystalline form and its chemical properties had been studied.[267] Continued investigation concerning its isolation and chemical properties showed that the compound, which in its pure form was practically insoluble in sodium carbonate and was highly insoluble in alcohol, was easily soluble in aqueous sodium carbonate and in 95 per cent alcohol when it was present in partially purified form. When pure, the compound possessed feeble acidic properties and also apparently contained a hydroxyl group which reacted with metal alkalies, but not with carbonates. It contained an amino group and 65 per cent of the molecule was iodine. It formed derivatives apparently through the nitrogen and a mono-acetyl and ureide were prepared. The ultimate analysis of the compound and of its derivatives led to the conclusion that the substance was a tri-iodo derivative of 2-oxo-tetrahydro-indole-3-propionic acid.

One of the earliest hypotheses concerning the chemical nature of the active constituent of the thyroid suggested that it was an iodized tryptophane, and when the analyses and chemical properties so closely agreed with that of a tri-iodo-2-oxo-derivative of indole it was suggested by Kendall and Osterberg that the probable structure of this compound was 4,5,6,tri-iodo,2,4,5,6,tetrahydro-2-oxo-indole-3-propionic acid.

Since, at that time, this nucleus appeared to be a derivative of oxo-indole it was named "thyroxyindole." This was shortened to "thyroxin." This name was chosen because it was felt that the activity of the compound was not due solely to its iodine content and that the organic nucleus to which the iodine was attached carried the essential chemical groupings which were responsible for its physiological activity. ("Thyroxin" has been proved to be a basic substance essentially similar to tyrosine: it should therefore be spelled with final "e," thyroxine.)

Structural formula assigned for thyroxine by Kendall and Osterberg:



As soon as a method was available by which thyroxine could be separated from thyroid material, further work concerning the isolation of this compound was not carried out and further investigation of the chemical properties of thyroxine was not made except with small samples of material not exceeding 100 mg.

The separation of this compound from thyroid material secured in the United States was an expensive process; $350.00 a gram was not sufficient to cover the cost of isolation by the method employed. The cost of the material practically prohibited chemical investigations of the compound itself, and since it seemed probable that thyroxine was a derivative of 2-oxo-indole-3-propionic acid, a detailed investigation of this family of compounds was undertaken. This series of compounds proved to possess chemical properties which were both interesting and highly suggestive that thyroxine was a derivative of oxo-indolepropionic acid. It was therefore decided to investigate the influence of minor changes in the structure of various compounds of this series in the hope that, even though the original position of the halogen in the molecule was incorrect, some other tri-iodo derivative of 2-oxo-indole-3-propionic acid would prove to be thyroxine. During the years 1922 to 1926, all possible hydro derivatives of 2-oxo-indole-3-propionic acid were prepared and the stability of the halogen derivatives of this series of compounds provided encouraging evidence that some tri-iodo derivative would prove to be as stable as thyroxine to the action of alkali.

In 1924, Harington [199] undertook an investigation concerning the chemical nature of thyroxine. The expense of the material deterred this investigator from the use of thyroxine itself as it had Kendall and Osterberg, and his first attempt was to prepare tri-iodo-phenyl-pyrrolidone-carboxylic acid. After he had succeeded in the synthesis of this compound he showed that the substance was not identical with thyroxine.

This experience convinced Harington that it was essential to secure a large amount of thyroxine and to investigate the chemical properties of this substance through degradation products. Since the price of sufficient thyroxine to permit the preparation of degradation products was prohibitive, Harington [200] undertook an investigation with the isolation of a large amount of thyroxine as its objective. After unsuccessful attempts to digest the thyroid material with pepsin and trypsin he tried to use the method for the hydrolysis of the thyroid proteins with sodium hydroxide. With the hope of finding a more satisfactory treatment for the separation of thyroxine he treated desiccated thyroid with barium hydroxide and in a relatively short time he secured about 100 grams of crude material, which yielded about 35 grams of pure, crystalline thyroxine. Harington [201] reduced the iodine from thyroxine by the action of hydrogen in the presence of palladium and showed that for each equivalent of iodine removed from the molecule two equivalents of hydrogen were required. The resulting organic compound, desiodothyroxine, proved to have chemical properties which permitted its easy purification and separation in crystalline form.

IDENTIFICATION OF THYROXINE

Analysis of this material showed it to have the empirical formula, $C_{15}H_{15}O_4N$. The nitrogen was all present in the form of amino nitrogen; the molecule possessed one acid group and one hydroxyl group. Fusion with potassium hydroxide at 310° in an atmosphere of hydrogen produced *para*hydroxy benzoic acid, quinol, ammonia, and oxalic acid. On exhaustive methylation desiodo-thyroxine behaved as does tyrosine. The completely methylated product lost trimethyl amine when boiled with alkali and formed a derivative which was an unsaturated acid.

Methoxyl determination of this acid indicated one methoxyl group. When the unsaturated acid was oxidized with potassium permanganate it gave a neutral substance together with oxalic acid. The neutral compound proved to be an aldehyde which when further oxidized yielded an acid, $C_{14}H_{12}O_4$, stable to boiling alkaline permanganate. These results indicated the probable presence in desiodo-thyroxine of two benzene rings.

The reaction on exhaustive methylation showed with almost a certainty the presence of an amino acid. The presence of one methoxyl group in the unsaturated acid showed the presence of only one phenolic

group and oxidation with the formation of oxalic acid and a residual stable acid was evidence of the presence of a three carbon side-chain. The above reactions may be represented thus:

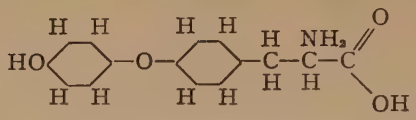
$$HO.C_{12}H_8OCH_2CHNH_2COOH \rightarrow CH_3OC_{12}H_8OCH:CHCOOH$$
$$CH_3OC_{12}H_8OCH:CHCOOH \rightarrow CH_3OC_{12}H_8OCHO \rightarrow CH_3OC_{12}H_8OCOOH$$

The remaining question was the character of the two benzene ring groups—$C_{12}H_8O$. The two benzene rings must be linked through an oxygen bridge and not through a carbon as no benzoquinone derivative was formed on oxidation. This structure which was tentatively assigned accounted for all the chemical properties of desiodo-thyroxine and in order to prove the correctness of the structure suggested, a substance possessing this formula was synthesized.

*Para*bromo-anisol was condensed with the potassium salt of *para*-cresol with the formation of 4(4′ methoxy phenoxy) toluene. This compound, after treatment with hydriodic acid, gave the corresponding phenoxy toluene, which proved to be identical with the substance obtained after mild potash fusion of desiodo-thyroxine. When this derivative of diphenyl oxide was boiled with permanganate it was converted into the methoxy phenoxy benzoic acid. The synthesis of these compounds which were identical with the degradation products of desiodo-thyroxine proved that the benzene rings were linked through oxygen. Barger has pointed out that, although synthetic derivatives of diphenyl oxide have been prepared, a member of this group of compounds has never been found as a natural product.

The preparation of this series of new substances was continued. *Para*bromo-anisol was condensed with potassium phenolate, giving 4-methoxy phenoxy benzene, and to this an aldehyde group was added *para* to the oxygen bridge. This aldehyde could be oxidized to the same acid which has been described. From the aldehyde it was possible to prepare the three carbon side-chain by two methods: First, the aldehyde was heated in the presence of acetic anhydride and sodium acetate with glycine anhydride. When the condensation product was boiled with hydriodic acid and red phosphorus it underwent a simultaneous reduction hydrolysis and demethylation with the formation of β[4(4′ hydroxy phenoxy)] phenyl α amino propionic acid, which was identical with desiodo-thyroxine. The second synthesis was carried out by condensing the 4(4′ methoxy phenoxy) benzaldehyde with hydantoin. The condensation product was boiled with hydriodic acid and red phosphorus

in the same way and desiodo-thyroxine was separated. This furnished complete proof of the structural formula of desiodo-thyroxine.



(Structure of *desiodo-thyroxine* assigned by Harington.)

Harington published his results after the completion of the synthesis of desiodo-thyroxine (see Fig. 2) and then returned to the problem which was now to synthesize the tetra-iodo derivative which had iodine in the same four positions as are substituted with iodine in thyroxine.

Within a year, Harington and Barger published the synthesis of the tetra-iodo derivative of desiodo-thyroxine and proved that this compound is identical with thyroxine. The synthesis of the tetra-iodo derivative of desiodo-thyroxine could not be accomplished by directly iodizing the latter compound. When this was tried, it was found that only two atoms of iodine could be attached to the nucleus of thyroxine.

A color reaction which was described by Kendall and Osterberg is given by thyroxine. Harington showed that this color is also given by *ortho*-iodohydroxyl derivatives of benzene, and because of this and from knowledge of di-iodotyrosine it was concluded that two atoms of iodine were attached to each benzene ring and that they were adjacent to the oxygen atoms.

Although it was easy to introduce the aldehyde group into the monomethyl ether of *para*hydroxydiphenyl oxide, this reaction could not be carried out when two iodine atoms were present in the benzene ring. The synthesis was finally completed through the action of monomethyl quinol on tri-iodonitro benzene. In the latter substance the iodine which occupies the position *para* to the nitro group is much more reactive than the other two atoms of iodine and it can be readily replaced through the action of the potassium salt of the monomethyl ether of quinol. The nitro group in this diphenyl oxide derivative was then reduced to an amine and the amine was replaced with a nitrile. Reduction of the nitrile with stannous chloride to an aldehyde was carried out and this aldehyde was then condensed with hippuric acid instead of with hydantoin, but otherwise the method used was the same as in the synthesis of desiodo-thyroxine.

The compound obtained by condensation with hippuric acid contained a double bond in the side-chain which required reduction. It

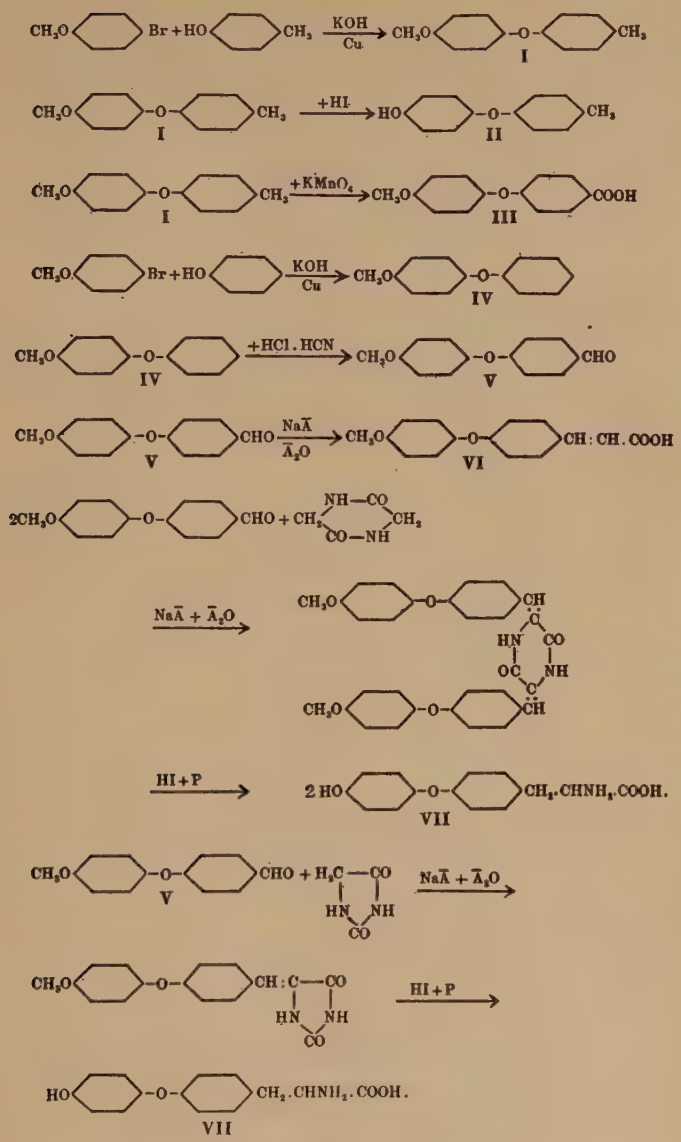


FIG. 2.—The constitution of desiodo-thyroxine.

was subsequently found that if this reduction was carried out with hydriodic acid in a solution of acetic anhydride a good yield was obtained, (Harington and McCartney). In addition to the reduction of the

double bond on the side-chain the methyl ether was removed from th hydroxyl group of the outer benzene ring and the benzoyl group wa hydrolyzed from the amine. The di-iodo compound which was obtaine

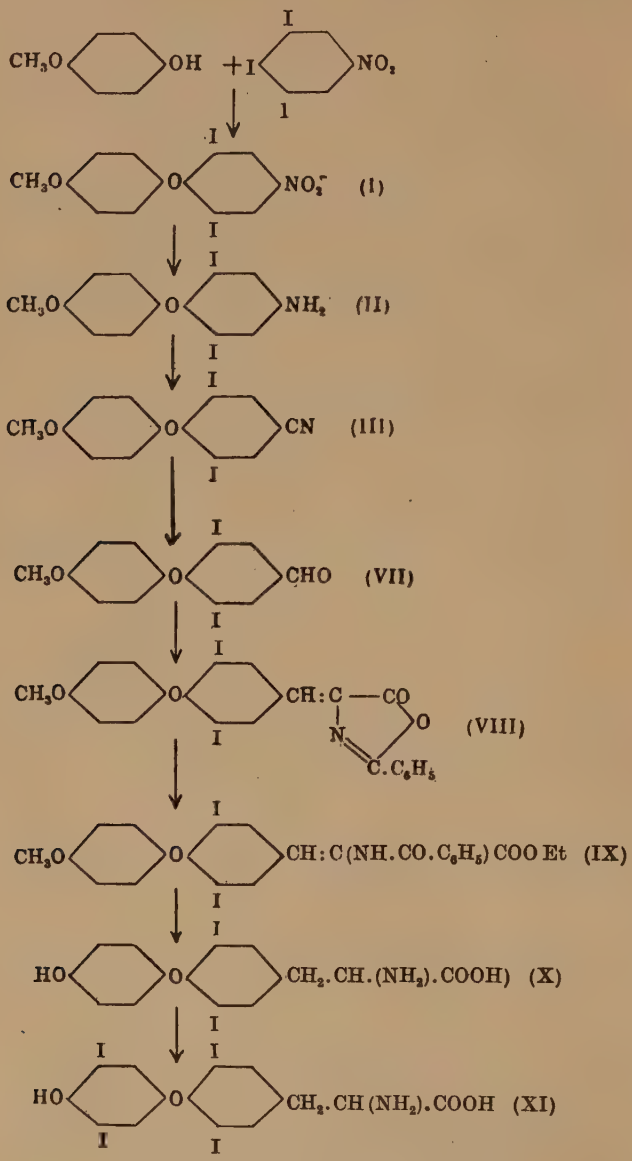


FIG. 3.—The synthesis of thyroxine.

readily took up two more atoms of iodine and a tetra-iodo derivative was obtained which was identified as thyroxine. (Fig. 3.)

Although its chemical proof was beyond question, the material was subjected to possibly the most difficult criterion for a substance to pass, namely, to produce the unique effect of thyroxine on the basal metabolic rate. The synthetic substance produced an increase in the basal metabolism which was entirely comparable with that produced by thyroxine itself. Lyon and Redhead,[320] and Lyon,[319] and Abderhalden and Hartmann.[3]

Kendall and Osterberg, in 1919, reported that thyroxine did not possess optical activity. This result was confirmed by Harington, and, after the formula was shown to be a tetra-iodo derivative of an oxyphenyl tyrosine, the reason for the absence of optical activity became evident. Prolonged treatment with sodium hydroxide undoubtedly produced the racemic form of the compound. The determination of the relative physiological activity of the optical isomers of thyroxine will be awaited with interest.

Besides the investigation of Kendall and Osterberg on the synthesis of the 2-oxo-indole-3-propionic acid series of compounds, which was undertaken in the hope of synthesizing thyroxine, other work was carried on with this same objective in Germany. Kalb, Schweizer, Zellner, and Berthold prepared a series of iodo derivatives of indole-2-carbonic-3-propionic acid.

Finally, mention of the isolation of tyrosine from thyroxine by Dakin should be made. By the use of strong hydriodic acid in a sealed tube at 140° Dakin was able to reduce the iodine and separate tyrosine. This was accomplished just at the time that the preliminary announcements were made of Harington's work. Dakin generously withdrew his manuscript which was in the hands of the editor of the journal, in favor of Harington.

SUMMARY

Hydrolysis of the thyroid proteins with alkali destroys most of the physiological activity of the gland; the fraction insoluble in acid, after alkaline hydrolysis, contains an iodine compound which possesses qualitatively all of the physiological activity of desiccated thyroid. This iodine compound, thyroxine, can be separated as the insoluble barium salt and it can be crystallized from alkaline alcohol by the addition of acetic acid. Although at first believed to be a tri-iodo derivative of oxo-indole-propionic acid, it has been shown to be a tetra-iodo derivative of oxyphenyl-

tyrosine. This was proved by identification of the degradation products of thyroxine and finally by showing that the physiological activity of a tetra-iodo-oxyphenyltyrosine prepared synthetically is identical with that of the natural product.

Chapter 3

Methods for the Separation of Thyroxine

Harington [200] suggested the following method for the separation of thyroxine from thyroid material: 500 grams of desiccated thyroid was treated with a 10 per cent solution of an equal weight of barium hydroxide. After about five to six hours' hydrolysis of the proteins, the solution was cooled and then filtered. The filtrate was a light yellow, clear solution, which contained compounds soluble in barium hydroxide but insoluble in acid. Acidification with hydrochloric acid produced a heavy, flocculent precipitate which settled to the bottom of the container. This was removed by filtration, dissolved in a small amount of ammonia, and treated with 40 per cent barium hydroxide, for eighteen hours. This solution was filtered hot, and the insoluble material was washed with hot water. The material soluble in 40 per cent barium hydroxide was discarded.

The fraction insoluble in the 40 per cent barium hydroxide was dissolved in a solution containing sodium hydroxide and sodium sulfate. It was then made acid with sulfuric acid and a heavy white flocculent material separated and settled to the bottom of the container when the solution was boiled. This material was dissolved in alkaline alcohol; when the solution was made acid with acetic acid, thyroxine separated in partially crystalline form.

The first step in this hydrolysis of desiccated thyroid with 10 per cent barium hydroxide resulted in the separation of a barium-insoluble fraction. This fraction was decomposed with sodium hydroxide and sodium sulfate, was precipitated with acid and treated with 40 per cent barium hydroxide. From the 10 per cent barium hydroxide soluble material from 500 grams of desiccated thyroid, Harington was able to isolate 400 mg. of thyroxine, and from the 10 per cent barium hydroxide insoluble material of 2000 grams of desiccated thyroid, he isolated 900 mg. more, giving a total yield of 0.125 per cent. The iodine in this amount of thyroxine was equivalent to 14 per cent of the total iodine contained in the desiccated thyroid.

The yield of thyroxine from the total amount of thyroid material used when my method for its isolation was being perfected [269] was 33 grams. This is a yield of about 0.0011 per cent. Since many samples of thyroid material during the investigation had failed to give any thyroxine it was difficult to explain the high yield obtained by Harington.

From the increased yield obtained Harington drew the natural conclusion that thyroxine is less stable in the presence of 5 per cent sodium hydroxide than it is in barium hydroxide. It therefore became desirable to investigate three questions in regard to the separation of thyroxine. The first was whether the use of barium hydroxide as described by Harington would furnish a satisfactory method for the isolation of thyroxine from all samples of thyroid material; the second, whether sodium hydroxide could be used for the isolation of thyroxine without destruction of this material in amount greater than that brought about by barium hydroxide; and third, whether thyroxine can be separated from all samples of desiccated thyroid which contain iodine.

An extended investigation in my laboratory was begun with barium hydroxide in the hope that the method could be used for the assay of the thyroxine content of thyroid material. Several samples of American desiccated thyroid were treated in the manner described by Harington. The material was desiccated, fat-free thyroid glands of hogs, which contained from 0.21 to 0.34 per cent of iodine. No thyroxine was obtained. Several other samples of American desiccated thyroid were treated as outlined by Harington, but thyroxine could not be isolated. However, from these same samples thyroxine could not be isolated by any other method.

Although entirely negative results were obtained with all samples of American desiccated thyroid investigated, it is possible that other samples may furnish a yield of thyroxine comparable to that given by the desiccated thyroid used by Harington.

Other difficulties were encountered. The solutions passed through a filter but slowly and they could not be centrifuged. This type of solution was frequently observed with samples of thyroid material which were prepared in the winter months. Experience with the use of barium hydroxide in the treatment of thyroid glands removed from animals grown in the United States emphasized the need of a method for the isolation of thyroxine from all samples of thyroid material, which would give the maximal yield of this iodine-containing compound.

Since barium hydroxide could not be conveniently used in the initial hydrolysis of all samples of desiccated thyroid I decided to carry out

the hydrolysis of the proteins with sodium hydroxide. By subjecting thyroxine and the acetyl of thyroxine to treatment with 5 per cent sodium hydroxide for twenty-four hours it was shown that the thyroxine could be recovered almost quantitatively. Iodine was not broken off, even when the solution was exposed to molecular oxygen.

The weight of the sodium hydroxide used in my original method was equivalent to 20 per cent of the weight of the fresh thyroid material, but more satisfactory results were obtained by reducing both the amount of sodium hydroxide and the volume of the solution. The method ultimately adopted is as follows: 2 kg. of flake sodium hydroxide is dissolved in 5 liters of water in a 35 liter nickel container. To this is added 22.5 kg. of fresh thyroid glands, and the total volume is made 30 liters. The solution is stirred for a few minutes until the alkali is thoroughly mixed with the glands. The nickel vessel is then heated without agitation by means of a boiling water jacket which surrounds it. The temperature is maintained between 95 and 100° for twenty-four hours, but the solution is not stirred after the initial thorough mixing with the alkali.

When the process is carried out in this way almost the entire amount of fat contained in the glands rises to the surface and can be separated without difficulty. It is not removed during the heating as it prevents evaporation of the water. After the solution has been heated for twenty-four hours it is drawn off through a siphon into a large stoneware crock containing 20 liters of water and 8 kg. of sodium chloride of technical grade. The solution is allowed to cool to room temperature; the layer of fat on the surface is then removed, together with the uppermost layer of the sodium soap of fatty acids, which was saponified during the treatment with alkali. A few kilograms of ice is added to the solution which is then made acid with commercial hydrochloric acid. Sufficient fatty acids are present to cause the precipitate to rise to the surface.

Separation of the insoluble material is made more complete by the sodium chloride resulting from the neutralization of the alkali and by the sodium chloride which was added. After three or four hours the solution is siphoned out of the crock, care being exercised that none of the flocculent precipitate is lost. The portion that remains in the crock is filtered through a suction funnel on a pad of infusorial earth.

The precipitate is placed again in the nickel container and dissolved in 10 liters of 5 per cent sodium hydroxide and the solution is heated for eighteen hours. From 400 to 500 grams of crystalline barium hydroxide, depending on the amount of fatty acids present, is dissolved in hot water

and added to the solution which is heated for three hours after the addition of the barium hydroxide. The barium hydroxide precipitates all of the fatty acids which are present and also carries out of solution a large amount of the black, tarry impurities.

Thyroxine is not precipitated by the barium at this point, because only a small amount of barium hydroxide remains not precipitated by the tarry material and the fatty acids, and also because barium hydroxide does not precipitate thyroxine in a solution containing products of hydrolysis and this concentration of sodium hydroxide. If an insufficient amount of barium hydroxide is added, the solution is difficult to filter, but if the tarry material has been completely precipitated as insoluble barium salts the solution filters easily on a large Buchner funnel.

The filtrate is made acid with hydrochloric acid which produces a small flocculent precipitate, A. This is allowed to settle to the bottom of the container for a few hours, and is then removed by filtration. By these two steps which are not time-consuming, the thyroxine is concentrated in a small fraction and from control experiments with English desiccated thyroid in which material has been treated both by Harington's method and by the method outlined, it has been shown that the loss of thyroxine from this hydrolysis is not significant. For the completion of the hydrolysis of thyroxine from the protein and in order to destroy the interfering compounds as much as possible, the precipitate is treated with a high concentration of barium hydroxide. In my original method [269] the partially purified thyroxine was repeatedly treated with 10 per cent barium hydroxide. The fraction insoluble in this solution was subsequently crystallized in the form of its sodium salt. Harington has suggested the use of stronger barium hydroxide solution.

The precipitate A, which has been described, is dissolved in a total volume of 500 cc. of water containing from 200 to 250 grams of crystalline barium hydroxide. Ammonia is used to assist the solution. After being heated from eighteen to twenty hours, the solution is filtered hot and the barium-insoluble material is decomposed with sodium hydroxide and sodium sulfate. The barium hydroxide solution is made acid with hydrochloric acid and the precipitate is redissolved in sodium hydroxide and reprecipitated to free it from barium. Thyroxine will not separate from a solution of this precipitate after it has been dissolved in dilute alkaline alcohol and has been made acid with acetic acid. With some samples of thyroid material, however, fractional precipitation with ammonium chloride may separate thyroxine which can be further purified and finally crystallized from alcohol.

Retreatment of the barium-soluble fraction with 40 per cent barium hydroxide may not separate any barium-insoluble fraction although the material is insoluble in acid. The compounds contained in this portion of the split products have the power to prevent thyroxine from separating in crystalline form. Even pure, crystalline thyroxine cannot be recovered from an alcoholic solution of the barium-soluble compounds. For this reason it is impossible to assume that all thyroxine which was present in the partially purified precipitate has been separated by treatment with 40 per cent barium hydroxide.

The sodium hydroxide sodium sulfate solution of the barium-insoluble material is made acid and a heavy, black, tarry precipitate separates from the solution. This material is dried and weighed.

In the description of the hydrolysis of thyroid proteins with barium hydroxide Harington [200] pointed out that the use of this alkali produces light-colored solutions which contrast favorably with the similar solutions of thyroid material secured by hydrolysis with sodium hydroxide. The appearance of the solutions and precipitates which are obtained by the use of sodium hydroxide is not encouraging, but a simple treatment has been found which results in an almost quantitative separation of the black, tarry impurities from the thyroxine and other colorless compounds which are present. This separation is based on the insolubility of the sodium salt of the black impurities in neutral 95 per cent alcohol. The details of the separation are as follows: One part of the dried acid-insoluble material precipitated from the solution of the barium-insoluble fraction in sodium hydroxide and sodium sulfate is dissolved in about 150 parts of 95 per cent alcohol which also contains a small amount of hydrochloric acid. Solution is complete. Finely powdered sodium hydroxide is now added from the end of a spatula with constant stirring. Each addition of the hydroxide causes a turbidity and the neutralization of the hydrochloric acid is continued until red litmus paper turns faintly blue. At this hydroxyl concentration the sodium salt of the black, tarry impurities is almost entirely insoluble and settles out as a brown precipitate. It is easily removed by filtration. The separation of the impurities is made more complete by the addition of 3 or 4 grams of sodium hydroxide and treatment of the solution with carbon dioxide. This removes the sodium hydroxide as carbonate or bicarbonate and further removes the brown impurities from the solution. The sodium carbonate and precipitated material are filtered out and the light-colored filtrate is acidified with acetic acid. This is heated on a steam bath for six to

eight hours under conditions of minimal evaporation. Thyroxine, if present, separates as a white, crystalline material.

Iodine determinations which have been carried out on all the successive solutions and precipitates have shown interesting variations in the distribution of iodine throughout the various steps, depending on the sample of thyroid used. For a quantitative separation of the thyroxine present it was found necessary to re-treat with barium hydroxide the sodium salt of the impurities which were insoluble in neutral alcohol and to re-treat the mother liquor after the first separation of thyroxine, although not more than from 200 to 300 mg. of thyroxine is recovered from retreatment of these two fractions.

By this method of isolation many different samples of thyroid glands have been investigated, both fresh and desiccated glands. With the object of investigating in more detail the conditions for the most economical isolation of thyroxine, a series of experiments was carried out in which the yield of thyroxine was determined from the same weight of hog thyroid glands secured throughout each month of the year. It was shown that in June, July and August the amount of thyroxine may be almost five times as much as the amount which can be isolated from the same weight of hog gland in January and February.

In addition to the amount of thyroxine, which varies with the sample of thyroid, there are other marked differences in the chemical constituents of the gland. Hydrolysis of American desiccated thyroid with barium hydroxide produces a light-colored solution which may be filtered from the barium-insoluble precipitate. The solution contains acid-insoluble material which closely resembles that obtained from similar treatment of English desiccated thyroid, but the barium-insoluble fraction is much larger and it cannot be satisfactorily decomposed with sodium hydroxide and sodium sulfate. The material does not consist of fatty acids, but other products are present which although soluble in sodium hydroxide form thick, gelatinous solutions.

When certain samples of desiccated thyroid prepared in England are treated in this manner the volume of material insoluble in barium hydroxide is small and it can be decomposed easily with sodium hydroxide and sodium sulfate. The alkaline solution thus obtained, when acidified does not give a heavy, bulky precipitate.

It was interesting to find that glands secured from hogs grown in the middle western states of the United States show a much smaller fraction insoluble in barium hydroxide in the summer months than they do in the winter. Detailed investigation of the material insoluble in

barium hydroxide has not been carried out, but the variations in the amount and its general chemical characteristics furnish evidence of changes in the chemical constituents of the thyroid gland which vary with the season and with geographical distribution in as marked a manner as does the thyroxine content.

Although it had been noted during the first years of this investigation that certain thyroid glands contained far more thyroxine than others and that in general more could be obtained in the summer months than in the winter, no quantitative investigation of this factor on the cost of production was made either by me or by the company manufacturing the material on a commercial scale. When, however, a satisfactory method for the assay of this material was worked out it became evident that the treatment of thyroid material from the months from October to June is not practical from a commercial standpoint. Since the cost of the raw material and the cost of treatment are the same throughout the year, it is evident that if only little thyroxine is obtained during half of the year the average cost of its isolation is greatly increased. By treatment of thyroid glands secured in the middle western states during the summer and autumn months thyroxine may be separated at small expense, and the price of the material may be reduced to about 20 per cent of its former figure. If the cost of synthetic thyroxine is high, it probably will be more economical to isolate the material from thyroid glands of animals either grown in England or in other places where the thyroxine content makes the separation practicable throughout the entire year, or else in the United States during the summer months.

These investigations of the thyroxine content have clearly indicated seasonal and geographical variations in the total iodine and the thyroxine content of thyroid material. (See Fig. 9 on page 56.) They furthermore indicate still another factor. This has been recognized too recently to permit a thorough investigation, but it now seems probable that the thyroxine in desiccated thyroid may undergo a chemical change which does not influence its physiological activity, but which makes impossible the separation of thyroxine in crystalline form. Evidence for this is the fact that at least traces of thyroxine could be separated from all samples of fresh thyroid material which have been examined. Even glands which were secured in January and February and which had only a small amount of iodine contained significant amounts of thyroxine. However, many samples of desiccated thyroid have been examined from which thyroxine could not be isolated although the iodine content was as high as 0.5 per cent. These results are highly suggestive that some chemical

reaction occurs in the desiccated thyroid which so alters thyroxine that it cannot withstand hydrolysis with alkali. Whatever this change may be it does not result in destruction of the physiological activity.

One conclusion which can be drawn from the events concerned in the separation of this compound is the benefit of an international attack on biological problems. If Harington had applied his method for the separation of thyroxine to certain samples of desiccated thyroid he would not have secured sufficient material to carry out the identification of thyroxine. At the time Harington undertook his investigation it was not known that the geographical factor was of importance and the fact that the amount of thyroxine which can be isolated in crystalline form has no relation to the total iodine of some samples of desiccated thyroid was not recognized. It had not been shown that in January the amount present is too low to make treatment of the material profitable. As a result of the work on this subject in both the United States and Great Britain our knowledge of the gland has increased not only concerning the chemical identification of thyroxine but also concerning many phases of its physiological activity and response to changes in the animal organism.

SUMMARY

Thyroxine may be isolated from the thyroid proteins either by hydrolysis of the material with 10 per cent barium hydroxide followed by a second treatment with 40 per cent barium hydroxide or the proteins may be treated first with 5 per cent sodium hydroxide and the acid-insoluble material re-treated with dilute barium and then with strong barium hydroxide. Thyroxine and the acetyl derivative of thyroxine are not destroyed with sodium hydroxide. There are marked seasonal variations in the thyroxine content of fresh hog thyroid glands removed from animals grown in the Mississippi valley. Some thyroxine can be isolated from samples secured during each month of the year. The amount separated in July may be five times the amount separated in January. Many samples of desiccated thyroid which possess typical physiological activity do not contain thyroxine which can be separated in crystalline form. This indicates a possible chemical alteration of the thyroxine molecule without coincident loss of physiological activity.

Chapter 4

The Chemical and Physical Properties of Thyroxine

The following chemical properties of thyroxine and its derivatives were reported in 1919 by Kendall and Osterberg. Thyroxine possesses striking chemical properties: it is insoluble in all organic solvents; it is easily soluble in the form of either its alkali or mineral acid salt in alcohol. Formic acid dissolves the compound probably with formation of a salt but it is highly insoluble in acetic acid. When precipitated from a hot, aqueous solution with sulfuric acid, it separates in the form of a sulfate which is soluble in alcohol. When precipitated with hydrochloric acid the hydrochloride salt is somewhat hydrolyzed; however, this salt can be made by dissolving thyroxine in a small amount of alcohol and water with hydrochloric acid. Crystals of the hydrochloride form when the alcohol is removed in a vacuum. A 0.6 *N* solution of hydrochloric acid will dissolve one part of thyroxine in 84,000 parts of acid. One part of thyroxine is soluble in 77,000 parts of *N* sulfuric acid. When the sodium salt of thyroxine is precipitated at 25° with dilute hydrochloric acid, the solution contains one part of thyroxine in 263,000 parts of water. When thyroxine is precipitated with carbon dioxide, one part is soluble in 815,000 parts of water.

Thyroxine forms two series of salts with alkali metals, one in which the carboxyl group only is combined in the form of a salt and the other in which both the carboxyl and phenol groups are combined with the cation.

Thyroxine is but slightly soluble in sodium or potassium hydroxide in the cold if the alkali is stronger than 15 to 20 per cent. In a mixture of 66 per cent alcohol and 33 per cent water containing 1 per cent of sodium hydroxide, thyroxine may be dissolved to the extent of about 4 per cent. It is still more soluble in hot solutions but on cooling it will separate as the dimetal derivative. Thyroxine is not readily soluble in dilute ammonia: concentrated ammonia dissolves about 1.8 parts of thyroxine in 100 parts of solution.

PREPARATION OF METAL SALTS

Monometal derivatives. One hundred mg. of thyroxine is dissolved in 150 cc. of 1 per cent sodium hydroxide, and carbon dioxide is bubbled through the solution until thyroxine is precipitated. The suspension is now heated until solution is complete. On cooling, the monosodium salt separates in crystal form. The separation of the monosalt is assisted by a high concentration of sodium salts and is hindered by too great a dilution. The preparation of the potassium salt is similar to that of the sodium salt. (Fig. 4.) If the monometal salt is filtered on a Buchner

FIG. 4.—The monopotassium salt of thyroxine.

funnel and washed with 10 to 15 per cent sodium, ammonium, or potassium chloride, it is not dissolved through the paper. If washed with cold water, about 40 per cent is dissolved and passes into the filtrate and 60 per cent remains on the paper. This action is due to hydrolysis of the salt and liberation of the free carboxyl group.

The monosodium salt of thyroxine is soluble to the extent of about one part in 850 of 0.1 per cent sodium carbonate at 20°. One part of the monosodium salt dissolves in 570 parts of 1 per cent sodium carbonate at 23°.

The di-alkali metal derivatives. One hundred mg. of thyroxine is dissolved in 20 cc. of dilute sodium hydroxide and to this is added 150 cc.

of a solution of 10 per cent sodium hydroxide containing 20 per cent of sodium chloride. If a precipitate appears the solution is warmed and the clear solution is then allowed to stand until cold. The exact concentrations of sodium chloride and hydroxide are not important. The disodium derivative readily separates. If the strongly alkaline solution is decanted and replaced with 15 per cent sodium chloride solution, the crystals may be separated from the solution on a Buchner funnel. They are insoluble in 10 to 15 per cent sodium chloride and may be washed and dried. The dipotassium derivative is prepared in a similar way.

FIG. 5.—The silver salt of thyroxine.

The alkaline-earth salts of thyroxine. The barium, calcium, and magnesium salts of thyroxine are prepared by dissolving 50 to 100 mg. of thyroxine in 100 cc. of dilute sodium hydroxide with as small an amount of alkali as possible. The solution is heated to boiling and 20 cc. of a 20 per cent solution of barium, calcium, or magnesium chloride is added to the hot solution of thyroxine. Carbon dioxide is excluded. The magnesium and calcium salts are very slightly soluble in boiling water; 500 cc. of boiling water dissolves between 100 to 150 mg. of the barium salt. The barium salt may be suspended in 500 cc. of boiling water and the solution filtered through a Buchner funnel into a suction flask. If

30 cc. of 20 per cent barium chloride is added to the filtrate, the barium salt separates in crystalline form. Only a negligible amount of thyroxine is soluble in the presence of this amount of barium chloride. The barium salt under these conditions is unstable and will slowly turn pink; there is a slow decomposition and the salt becomes yellowish gray.

Silver, copper, nickel and zinc salts of thyroxine. The silver, copper, nickel, and zinc salts are prepared as follows: 50 to 100 mg. of thyroxine is dissolved in 25 to 50 cc. of concentrated ammonia. Twenty-five cc. of a 10 per cent solution of silver nitrate, copper sulfate, nickel sulfate, or zinc sulfate is made ammoniacal with strong ammonia so that the precipitated hydroxide is just carried into solution, and there is a slight excess of ammonia. The solution of the metal is now added to the ammoniacal solution of thyroxine; after standing a short time crystals of the metal salt separate. (Fig. 5.) If a large volume of ammonia is used, a larger amount of the solution of the metal may be required to start the crystallization. The silver, copper, nickel, or zinc salts suspended in water or dilute ammonia are soluble on the addition of sodium hydroxide solution.

THE ACTION OF NITROUS OXIDE ON THYROXINE

Thyroxine will react with nitrous acid: all of the nitrogen is liberated. In addition to this action of nitrous acid a characteristic color reaction is produced. A few milligrams of pure thyroxine, dissolved in 5 cc. of alcohol with three to four drops of 50 per cent hydrochloric acid, will give a yellow solution after the addition of five or six drops of 1 per cent sodium nitrite solution. This color is intensified by boiling. If the solution is cooled and concentrated ammonia is added until distinctly alkaline, a pink color is produced. This is a sensitive test for thyroxine: a distinct color is produced by one part of thyroxine in 40,000 parts of solution. If acetic or sulfuric acid is substituted for hydrochloric acid, the yellow is not so deep and the addition of ammonia produces not a pink, but a yellowish-orange. If the sample of thyroxine is impure, a yellow instead of a pink is produced with ammonia.

THE ACETYL OF THYROXINE

The acetyl of thyroxine is prepared by treatment of the sodium salt of thyroxine, in alcohol, with acetic anhydride. Sodium hydroxide is added until the nitrous acid color reaction is very faint, which indicates the formation of the di-acetyl derivative of thyroxine. Alcohol is then removed from the solution and the acetyl group attached to the hydroxyl is removed by treatment with sodium hydroxide.

Addition of strong sodium hydroxide precipitates the sodium salt of the acetyl. This is separated from the solution and redissolved in water, and is then recrystallized by the addition of sodium chloride. The sodium salt is dissolved in alcohol and poured into boiling dilute hydrochloric acid, which precipitates the acetyl derivative in pure, crystalline form. (Fig. 6.)

This derivative of thyroxine gives the same color reaction with nitrous acid and ammonia as does thyroxine, and the fact that further treatment with acetic anhydride prevents the development of color with

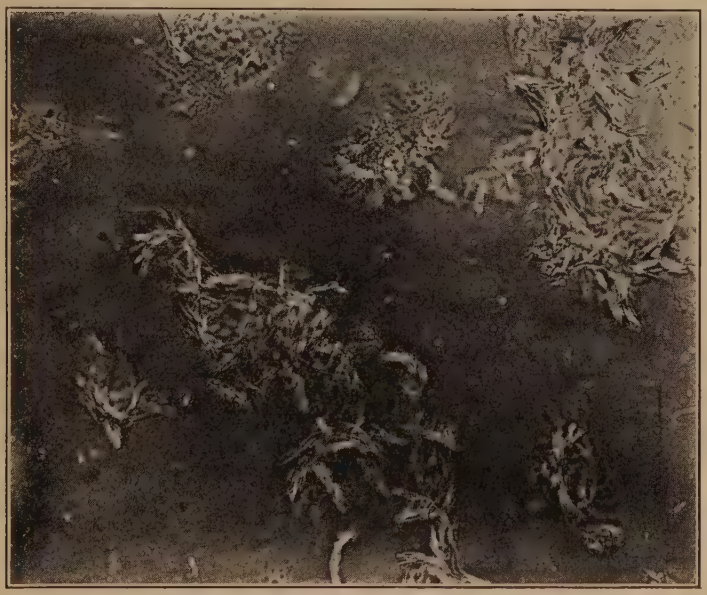


FIG. 6.—The acetyl of thyroxine.

nitrous acid indicates that the phenolic group on the outer benzene ring of thyroxine is essential for the color reaction.

The acetyl derivative is no more soluble in water than is thyroxine, but it is easily soluble in alcohol.

The acetyl group is hydrolyzed from the amine with great difficulty.[275] When this derivative is heated at 100° for six hours, in 5 per cent sodium hydroxide, not more than 25 per cent of the acetyl group is removed. When it is heated for eighteen hours in 5 per cent sodium hydroxide, the acetyl group is hydrolyzed and almost 90 per cent of the

thyroxine may be recovered. Glacial acetic acid saturated with dry hydrochloric acid gas will hydrolyze the acetyl from thyroxine when the solution is heated for sixteen hours in a sealed tube. The recovery of thyroxine in this case was about 85 per cent.

When the acetyl derivative was heated in a 40 per cent solution of barium hydroxide for twenty-four hours, over 90 per cent of the acetyl was recovered unchanged. The barium salt of the acetyl derivative is insoluble in 40 per cent barium hydroxide and the compound was precipitated out of the solution for the most part.

It is significant, however, that the acetyl group was not removed to any measurable extent by this concentration of barium hydroxide. This observation raises the question whether the amine group of thyroxine is substituted when the compound functions in the animal organism. Thyroxine can be separated from the proteins of the thyroid by barium hydroxide alone; this indicates that, if some group substitutes the amine it is more easily removed from the amine than is the acetyl group.

The acetyl derivative of thyroxine possesses one chemical property which is not shared by its mother substance. The sodium salt spontaneously decomposes in a neutral solution. When the acetyl of thyroxine is dissolved in alcohol containing acid, sodium hydroxide may be added to the solution without the destruction of any of the acetyl; however, if the solid crystals of the acetyl of thyroxine are added to alkaline alcohol the substance decomposes with the production of a yellow solution. If the sodium salt is crystallized from an aqueous solution by the addition of a high concentration of sodium chloride, and the crystals are filtered and washed with neutral sodium chloride solution, they are unstable and will spontaneously break down with the liberation of iodine. This reaction has not been explained and whether or not it is related to the oxidation of the molecule with molecular oxygen or whether it is related to the physiological action of thyroxine has not been shown.

THE UREIDE OF THYROXINE

The ureide of thyroxine is prepared by the addition of the sodium salt of thyroxine to glacial acetic acid which contains potassium cyanate. The ureide is soluble in the acetic acid. If the acetic acid is removed in a vacuum, the ureide may be purified through its sodium salt. If an alcoholic solution of the ureide is poured into boiling dilute hydrochloric acid the ureide crystallizes from solution. (Fig. 7.)

MELTING POINT OF THYROXINE

The melting point of thyroxine is markedly influenced by the rate of heating. If the melting point tube is maintained at as low a temperature as 210° for thirty minutes, darkening occurs, and the thyroxine will melt after long heating. If the melting point tube is heated at the rate of 10° a minute, the melting point is 250°. If the tube is heated at the rate of about 3° a minute, the melting point is between 230 and 235°.

FIG. 7.—The ureide of thyroxine.

Iodine is evolved when thyroxine melts, irrespective of the temperature. All the derivatives of thyroxine melt at a much lower point.

OTHER FORMS OF THYROXINE

One of the most interesting problems concerned with the structure of thyroxine is the product formed by precipitation of thyroxine from an alkaline solution with carbon dioxide. A very finely divided precipitate is produced, which under a high-powered microscope is shown to consist of fine hair-like crystals, curved, and matted together. Precipitated in this form thyroxine does not at all resemble the crystals obtained when this substance is separated from boiling solution with sulfuric, hydrochloric, or acetic acid.

Kendall and Osterberg assumed that the precipitate obtained through the action of carbon dioxide was a carbonate of thyroxine. It now seems improbable that a tetra-iodo derivative of oxyphenyltyrosine could form a carbonate and the most probable structure is a hydrate of thyroxine. It has been shown that when thyroxine is precipitated with carbon dioxide it is far more sensitive to the action of light. Crystals of thyroxine precipitated with carbon dioxide will turn yellowish-brown even when kept in a desiccator in a darkened cupboard.

When thyroxine is precipitated from a dilute alkaline solution through the addition of ammonium chloride, thyroxine also separates in a form which is not identical with that obtained by crystallization from alcohol or acetic acid, and Kendall and Osterberg suggested that this was a hydrated form of thyroxine; it is soluble in alcohol and has a much lower melting point than thyroxine.

OXO-INDOLEPROPIONIC ACID DERIVATIVES

The formation of apparently hydrated derivatives and the fact that thyroxine gave a test for indole when fused with sodium hydroxide suggested that the chemical reactions involved might be explained by the opening and closing of the lactam ring in an indolinone derivative. The chemical properties of the substance strengthened the hypothesis that the compound was a tri-iodo derivative of 2-oxo-indole-3-propionic acid. Although detailed defence of this formula is not at this time necessary it is a matter of interest to record the evidence on which several years' work was based.

The stability of certain halogen derivatives of the 2-oxo-indole-3-propionic acid series of compounds was not only striking, but conclusively showed that the stability of the halogens depended on the arrangement of the double bonds in the molecule. It therefore seemed probable that the positions of the iodine and the double bonds in thyroxine were such that the molecule as a whole was stable to alkali and mild oxidation. The formula originally assigned was excluded by synthesis, but the formula then suggested in which two double bonds lie in the cyclohexadiene ring was not excluded.

In confirmation of the hypothesis that thyroxine was an indole derivative, Hicks [221, 222] secured evidence obtained from the absorption-spectrum in the ultra-violet which suggested that thyroxine was indeed a member of the indole group of substances; furthermore, subjection of the synthetic products of the oxo-indolepropionic acid series to similar tests showed that the absorption-spectrum of these compounds agreed

quite closely with that of thyroxine, of iodo-isatin and of other related compounds. A satisfactory explanation of these results is not at hand, but it suggests that too great weight cannot at the present time be placed on the absorption spectra of organic compounds. Further investigation of the absorption-spectra of these substances and of thyroxine is desirable.

The details of the synthesis of the series of compounds belonging to the oxo-indolepropionic acid series need not be given in this monograph. The data are available. Suffice it to say that all possible configurations of the hydro and halogen derivatives were prepared and many interesting relationships established.

Just before the investigation of this series of compounds was completed Harington published his work concerning the identification of the nucleus of thyroxine to which the iodine is attached, and submitted conclusive evidence that the molecule is a derivative of tyrosine.

The work of Harington brought into question the analytical results on which were based the percentage composition and the formula of thyroxine. It became immediately evident that the percentage of carbon, hydrogen and oxygen and iodine in a substance containing 11 atoms of carbon and 3 atoms of iodine, agreed within experimental limits with the percentages of the carbon, hydrogen, oxygen, and iodine in a substance containing 15 atoms of carbon and 4 atoms of iodine. This striking coincidence, and the duplication by Harington of the published results obtained by Kendall and Osterberg on the determination of carbon, hydrogen and iodine showed that a significant error had not been made in the determination of these constituents. The difference between the results of Kendall and Osterberg and those of Harington, therefore, may be reduced to the fact that the molecular weight of thyroxine determined through the difference in the iodine content of this substance and three of its derivatives, the acetyl, ureide and sulfate gave the value of 585, and not 777. An explanation of this discrepancy was sought and has now been found.

The two derivatives, the acetyl and the sulfate of thyroxine, were again prepared in pure, crystalline form and were analyzed for iodine, by Kendall's method which had been formerly employed. The original results were obtained. The two sets of determinations did not differ by more than 0.10 per cent.

Analysis of these two compounds for carbon and hydrogen gave a result which agreed, within experimental limits, with the formula suggested by Harington and these figures also agreed satisfactorily with the formula based on the molecular weight 585. Determination of iodine

in these two derivatives was then made by the Carius method, and these results showed the iodine content to be that of a compound having a molecular weight of 777. It therefore is apparent that the value 585 for the molecular weight was obtained because of volatilization of traces of organically-bound iodine from the alkaline fusion of thyroxine. The determination of iodine in aqueous solution, by oxidation of the iodine to iodic acid, is accurate to a high degree; however, it is evident that if traces of organically-bound iodine are volatilized from the alkaline fusion the determination will be low.

Other examples of volatilization of traces of iodine and bromine have now been found in the 2-oxo-indole-3-propionic acid series of compounds: the 5,7-di-iodo derivative of this compound in which a hydroxyl group is attached to Carbon 3 may lose from 0.2 to 2 per cent of its iodine when fused with sodium hydroxide.

Whether or not organically-bound iodine is volatilized during the fusion with alkali is determined by slight alterations in the chemical configuration. Although the derivative of 5,7-di-iodo-2-oxo-indole-3-propionic acid with a hydroxyl group on Carbon 3 decomposes with liberation of 2,4-di-iodo-anilin, 5,7-di-iodo-indole-3-propionic acid itself does not lose iodine when fused with sodium hydroxide.

Harington,[200, 201] and Harington and Barger determined iodine satisfactorily by the same method as was used by Kendall and Osterberg to determine the iodine content of thyroxine and its derivatives.

The determination of the molecular weight of thyroxine through the analysis of this compound and its derivatives for iodine is now an unimportant detail of the investigation; however, it is essential to point out the limitations of the method for the determination of iodine and to emphasize the fact that certain iodine-containing compounds may be volatilized from the fusion melt. In the case of thyroxine it is remarkable that the percentage of the total amount which was volatilized was so uniform with several different derivatives.

The delay in the identification of the true chemical nature of thyroxine must be almost wholly attributed to the traces of organically-bound iodine which were volatilized from the alkaline fusion.

SUMMARY

Thyroxine forms mono and dimetal series of salts and the amine group of thyroxine forms salts with mineral acids. It is exceedingly insoluble in all organic solvents. The mono-alkali metal salts are slightly soluble, the di-alkali metal salts are easily soluble to the extent of

about 4 per cent. The alkaline earth, the silver, copper and zinc salts are insoluble. Nitrous acid liberates all of the nitrogen from thyroxine. Thyroxine gives a color reaction, which is sensitive to about one part in forty thousand, when alkali is added to the reaction product formed with nitrous acid. Mono and di-acetyl derivatives and a ureide of thyroxine may be prepared. The acetyl spontaneously decomposes with liberation of iodine in a neutral solution. There is a coincidence between the percentage composition and the absorption spectrum of thyroxine and triiodo-oxo-indolepropionic acid. The chemical properties of the halogen derivatives of hydro-oxo-indolepropionic acid indicated that thyroxine was a member of this series of compounds. The molecular weight of thyroxine was determined to be 585 and not 777 because of the volatilization of organically-bound iodine during the fusion with alkali.

Chapter 5

The Iodine Content of the Thyroid Gland

Since the publication by Baumann of the discovery that iodine is a normal constituent of the thyroid gland many analyses of thyroid glands of different animals, and of human beings have been reported. Roos [483] and Baumann [34] analyzed the thyroids of various animals and of human beings in several cities in Germany. Oswald [377] estimated the thyreoglobulin content of various glands from the determination of the iodine content of the glands, and compared the iodine and iodothyreoglobulin content of sheep and hog thyroids. He showed that iodothyreoglobulin made up the largest part of the colloid material. Thomas and Delhougne found that with suitable methods it is possible to demonstrate the presence of iodine in all new-born children. Fenger [153] showed that both normal and enlarged fetal glands contained iodine during at least the last few months of fetal life. Maurer and Diez [346] found iodine in fetal glands. Maurer [345] has continued this investigation and has shown the presence of from 37 to 1400 γ * of iodine for each 100 grams of fresh fetal thyroids of children seven to nine months old. He believes that the iodine compound in the fetus is present in an active form and that it parallels and supplements the action of the mother's gland. Abelin [6] has recently reported the presence of iodine in the fetal glands of calves and human beings. On the other hand, Herzfeld and Klinger [218] make the surprising statement that from a third to a half the thyroid glands of all persons that they examined do not contain iodine, or only traces, and they suggest that there is need to improve the type of food in order that the thyroid glands of persons living in Holland and Switzerland may have a sufficient amount of iodine.

Although there can be no question concerning the need of more iodine in the food, it seems probable that the absence or minimal content of iodine reported in such a high percentage of glands can be explained by faulty technic in the determination of iodine. These results do not conform to any of the other determinations of iodine which have been made by methods shown to be accurate.

* γ = 0.001 mg.

Cameron [85, 87] showed that a large amount of iodine is present in the thyroid gland of certain fish. He [86, 88] published a good review of the distribution of iodine and included the analyses of many plants and animals. Hunter and Simpson [244] reported the maximal amount of iodine found in the thyroid gland. They analyzed the glands of sheep grown in the Orkney Islands, and found 1.05 per cent of iodine. The high percentage of iodine in the gland was doubtless due to the high iodine content of the seaweed in their diet. This hypothesis was confirmed by feeding potassium iodide to sheep: it was found that the iodine content of these glands increased to 1.15 per cent. Certain thyroid glands of sheep received in my laboratory that had been fed potassium iodide for about nine months showed an iodine content of more than 1 per cent.

Arnold and Gley found that a goat's thyroid gland contained an average of 6 mg. of iodine; minimum, 1.93, maximum, 8.06. No relation was found between the iodine content and the weight of the gland. There was a noticeable difference in the iodine content of the two lobes. These goats came from the Mediterranean region.

Zunz determined the iodine content of the thyroid glands of man in relation to the ages of a series of patients who died of pneumonia. Cameron states that the maximal store of iodine for each gram of thyroid material is relatively constant. For most mammals, thus far examined, this amount is about 5 mg. for each gram of dried thyroid. The average normal iodine content of the thyroid gland of man has been reported to be 2 mg. for each gram of dried substance, and the maximal total store of iodine in the strictly normal gland does not exceed 25 mg. As Marine pointed out, the determination of the actual amount of iodine in the normal thyroid is of the utmost importance when iodine treatment is undertaken in goitrous regions. Other observers have found exceedingly high amounts of iodine in the thyroid glands of patients treated with iodine.

Marine and Rogoff [340] found that the iodine content of the thyroid gland of a dog may be increased several hundred per cent within five minutes by an injection of 50 mg. of potassium iodide into the femoral vein. As much as 18 per cent of an administration of 38 mg. given to a dog by mouth was recovered from the thyroid gland, whose ratio to body weight was 1:687. These figures emphasize the unique power of the thyroid gland to accumulate iodine when injected in large amounts. The observation that the thyroid glands of sheep and hogs can accumulate large amounts of iodine on a diet containing the merest traces of iodine, is equally striking and significant.

Smith [457] has analyzed goiter glands and shown the retention of iodine by the thyroid gland. Van Dyke [518] has shown that free iodine and sodium iodate cause only a slight increase of iodine in the gland. Thyroxine causes practically none, but potassium iodide is markedly absorbed.

Seidell [447] investigated the seasonal changes in the iodine content of the thyroid glands of sheep, dogs and other animals. He mentions some earlier work reported by Koch, in which it was stated that the glands contained three times as much iodine in winter as in the summer months. Although the glands examined by Seidell did not show a seasonal varia-

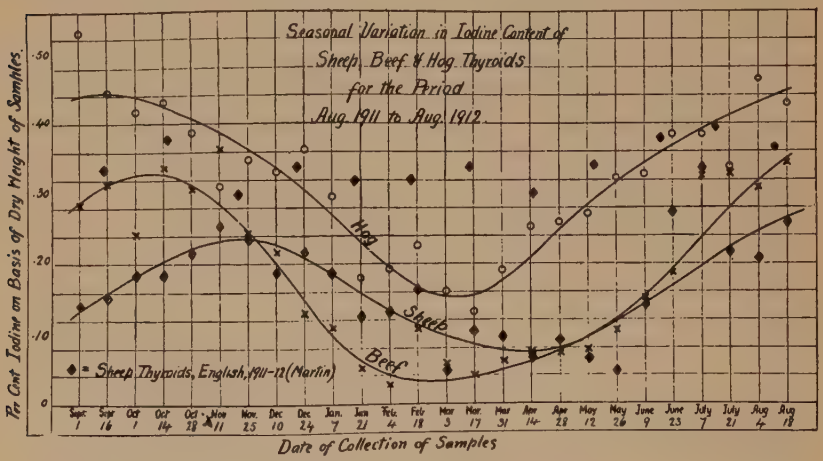


FIG. 8.—The seasonal variation in the iodine content of the thyroid gland (Seidell and Fenger).

tion, Seidell and Fenger [448] were able to show very striking seasonal fluctuations in the amount of iodine in the thyroid glands of hogs, sheep and beef. Fenger [154] has confirmed the iodine determinations of Seidell and Fenger and has again shown a seasonal variation in hog, sheep and beef thyroid glands. (Fig. 8.)

Martin [343] reported a similar investigation with sheep glands that were secured at Newcastle. The sheep were all raised within fifty miles of Newcastle, and were therefore probably at least moderately well supplied with iodine in the food. (See Table 1.)

Since Fenger has shown a definite seasonal variation of the iodine in the thyroid gland it is difficult to explain the results reported by Martin and by Guyer. The values are not high; therefore the diet prob-

TABLE 1. *Iodine in Desiccated Thyroid Glands of English Sheep (Martin).*

1911	Per Cent of Dry Gland
July	0.40
August	0.36
September	0.34
October	0.38
November	0.30
December	0.34
1912	
January	0.32
February	0.32
March	0.34
April	0.30
May	0.34
June	0.38

ably was not rich in iodine. The results are too consistent to be explained by faulty technic in the determination. It is hoped that with our increased knowledge of the relation of iodine to the activity of the gland, more work of a similar nature will be carried out in England and Scotland.

Guyer reported the analysis of Scottish sheep to be as follows:

TABLE 2. *Iodine in Desiccated Thyroid Glands of Scottish Sheep (Guyer).*

	Per Cent of Dry Gland
December	0.258
January	
February	0.222
March	0.266
April	0.229
May	0.251
June	0.279

In order to study seasonal variations in the total amount of iodine and thyroxine in the thyroid gland I have recently carried out an investigation in which 45 kg. of fresh thyroid glands of swine collected during the first few days of each month of the year, were treated for the isolation of thyroxine. These results have again demonstrated the seasonal variation pointed out by Seidell and Fenger, and are in contrast to the results obtained by Martin for the iodine content of thyroid glands removed from sheep grown in England. (Fig. 9.)

THE IODINE CONTENT OF THE TISSUES

In addition to the determination of iodine in the thyroid gland a large amount of work has been carried out in an attempt to determine the iodine content of blood, tissue and other body fluids. The earlier

results were misleading due to the limitations of the methods which were used. Occasionally iodine was reported in large amounts in tissues which were later shown to contain only traces, and on the other hand, many investigations indicated an entire absence of iodine from tissue and blood.

In 1920 Kendall and Richardson [274] showed that if large amounts of

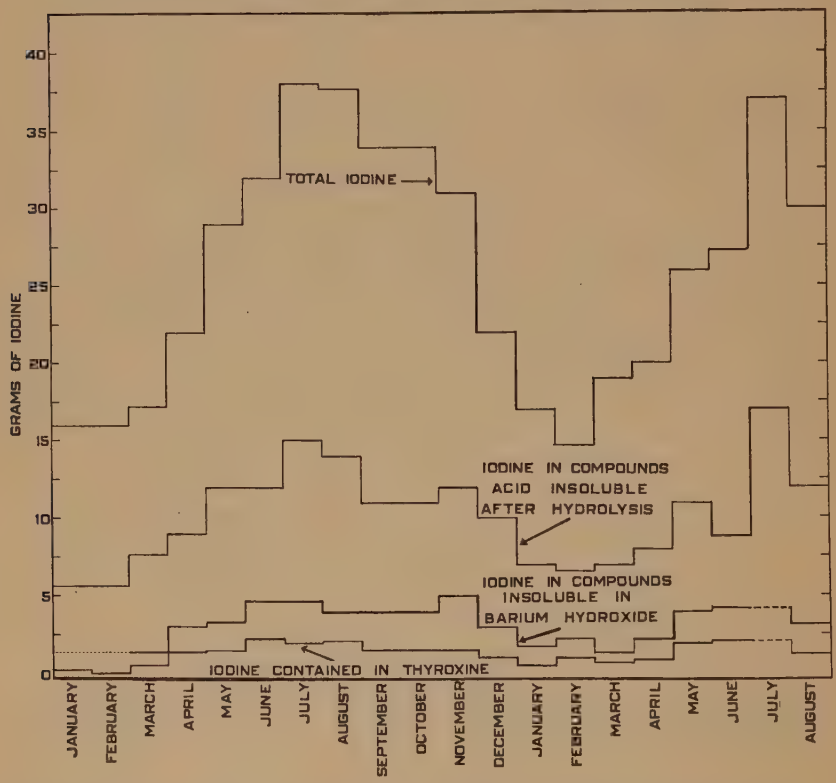


FIG. 9.—Seasonal variations of the iodine-containing compounds in various fractions obtained by hydrolysis of the thyroid with alkali (Kendall and Simonsen).[275]

tissue were taken the iodine could be determined with a high degree of accuracy and it was found that the blood of man, dog, ox, sheep and cat contained approximately 10 to 13 γ for each 100 grams of blood ($1\ \gamma = 0.001$ mg.). Subsequent investigators, notably Eggenberger and Fellenberg,[150] have modified the method of Rabourdin and of Baumann in which the iodine was determined by its color in chloroform and have shown that if the iodine is concentrated in a small volume of

chloroform it can be determined although present in extremely small amount.

In 1925, Veil and Sturm reported an extensive investigation of the total, the lipoid and the inorganic iodine in blood and tissue with Fellenberg's method. They have confirmed the amount of iodine found in blood to be approximately 10 to 12 γ for each 100 grams in summer and they found 8.3 γ to be the iodine content in the winter months. After administration of potassium iodide the maximal concentration in the blood was 1/385 of the total amount, and was found one and a half hours after it had been given. Sixty-eight per cent was excreted in the urine. The maximal concentration was reached at the end of two to three hours. If thyroid material was administered the maximal amount of iodine in the blood was one-third the total three hours after administration. The maximal amount of iodine in the urine was present from four to six hours after the administration: 33 per cent of the iodine in the urine was inorganic and 67 per cent was organic.

Fellenberg [151] showed that after administration of thyroid material 17 per cent of the iodine present was excreted in the first twenty-four hours and 4 per cent was excreted in the second twenty-four hours. By means of a dog with a bile fistula, I^{270} showed that when 200 mg. of thyroxine was injected 43 per cent was excreted in the bile during the following fifty hours and not more than 13 per cent of the total iodine was excreted in the urine. Krayer [294] has continued this study and shown that if 0.8 mg. of thyroxine was injected intravenously in the rat it was taken up by the liver and excreted through the bile in the intestine. After five to six hours 50 per cent was excreted in the feces; the excretion continued during the next day. Iodine was not found in the urine. Five days after the injection the muscle, bone and skin still were found to contain iodine in greater than normal amount although the thyroid gland did not contain a noticeable increase. When iodothyreoglobulin was administered it was also excreted in the bile, but in addition a portion of the iodine was found in the urine: iodine was also found in the urine after administration of di-iodotyrosine. The elimination of iodine, therefore, following iodothyreoglobulin, agreed in part with the elimination of iodine after the administration of di-iodotyrosine.

After an injection of thyroxine, small amounts were found to remain stored in the body for a very long time. This observation is in keeping with the long time during which thyroxine influences physiological processes. No explanation of the mode by which thyroxine functions was

secured through determination of the distribution of the compound in the body of the rat.

Maurer, Ducrue, and Palasoff have determined the iodine content in various organs of women and tissues of the human fetus. (See Table 3.)

TABLE 3. *Iodine Content (in γ* Per Cent) of Various Organs of Adult Women (Maurer, Ducrue and Palasoff).*

Age	Heart	Liver	Spleen	Adrenal	Ovary
35	66.2	90.6	....	136	520
30	....	38.5	54.0	157	250
45	67.0	52.0	33.2	62	172
40	63.3	16.6	....	95	496
23	....	80.0	120.0	...	2509
65	14.0	62.0	37.0	...	500
Average	52.6	56.6	61.0	112	741

Iodine Content (in γ Per Cent) of Various Organs of the New-Born.*

Age Fetal Months	Heart	Liver	Spleen	Thymus	Ovary	Thyroid
9	13.0	3.1	32	13.0	107.0	37
9	10.0	4.9	37	20.0	....	40
9	22.0	...	18	...	140.0	...
9	...	...	..	40.0	84.2	352
9	7.5	...	..	16.6	....	111
9	2.8	20.0	..	47.0	....	93
9	11.0	7.5	..	134.0	222.0	142
8	...	15.0	..	28.0	....	102
8	9.0	20.0	..	25.0	....	181
8	11.9	...	..	...	....	125
7	...	12.3	..	...	....	1400
7	30.6	45.0	..	90.0	....	170
Average	12.0	17.0	29	46.0	138.0	250

* 1 γ = 0.001 mg.

These results are evidence that appreciable amounts of iodine are present in all the tissues and although the amounts are extraordinarily small the presence of iodine and presumably of thyroxine must be considered in relation to the chemical reactions which are responsible for physiological activity. Veil and Sturm have considered iodine compounds in the blood which are soluble in alcohol and insoluble in chloroform to be inorganic iodides. Hudson has shown that the iodine content of the blood increased after thyroidectomy. Administration of fresh thyroid gland brought about a return to the normal iodine content. When thyroid feeding was discontinued the iodine content was again increased in the blood. Kraft has recently shown that there is a distribution of injected potassium iodide between the plasma and the corpuscles. The type of distribution

was independent of the concentration added and was the same in various animals of the same species. Experimental hyperthyroidism and thyroidectomy were without influence on the distribution of potassium iodide. If appreciable amounts of inorganic iodides are in the tissues in addition to the minute amount of thyroxine, then the concentration of this iodine-containing compound is less than the amount indicated by the determination of the total iodine. Its potency is correspondingly increased.

It now appears to be possible to determine the iodine content of biological material when it is present in extremely small amounts. Further work in this field may result in definite proof that the concentration of thyroxine present in various forms of hyperthyroidism is related to the severity of clinical symptoms.

SUMMARY

Iodine is found in the thyroid gland of all animals and its presence in fetal glands may be shown by methods which are sufficiently sensitive. The maximal iodine content of thyroid glands has been found to be about 1.2 per cent. The thyroid gland rapidly takes up iodine when it is injected in the form of potassium iodide. Eighteen per cent of 38 mg. was found in the thyroid gland whose ratio to body weight was 1 : 687. There is a marked seasonal variation in the iodine content of thyroid glands of animals grown in the northern portion of the Mississippi valley. A seasonal variation has not been observed in English and Scottish sheep. The iodine content of blood is approximately 0.010 mg. for each 100 grams. The iodine distribution in the body following the injection of iodides and of thyroxine has been determined. Thyroxine is not excreted in the urine and none, or very little iodine, is broken off in the inorganic form. By the use of micro methods iodine has been shown to be present in all of the tissues of the body.

Chapter 6

The Chemical Nature of Iodine Compounds in the Thyroid Gland

Whether the thyroid gland contains one or more iodine compounds is one of the most significant questions which has grown out of the chemical investigation of this gland. The thyroid proteins have been treated with various solvents to determine the effect on the distribution of the iodine-containing compounds. Romeis [424] extracted the gland with ether, alcohol, acetone and chloroform and tested the various fractions for biological activity. The increased rate of the metamorphosis of the tadpole was used as the criterion. He found that the iodine was divided between the various fractions and a definite separation could not be made, some activity being manifest in all the fractions.

Meyer fractioned the thyroid gland with ether, alcohol, water and acetone. Most of the iodine compounds remained in the residue. The iodine compounds soluble in water and insoluble in chloroform were called inorganic iodides. Twenty grams of dried thyroid glands contained 30.35 mg. of iodine: 1.72 mg. of iodine was alcohol-soluble; there was no inorganic or lipoid iodine; 7.4 mg. of iodine was in the protein material soluble in water, and there was 21 mg. of iodine in the residue. One hundred grams of fresh glands contained 27.8 mg. of iodine in the protein, 0.64 mg. in the lipoid, and 1.2 mg., inorganic. It seems probable that both the lipoid and inorganic iodine figures for fresh thyroid material are actually iodine organically-bound to protein which was soluble under the conditions of the experiment in the ether and chloroform, respectively. When the material was dried, no iodine compound was found in the chloroform or the ether extract.

A much more severe criterion of the form of combination is furnished by the hydrolysis of the entire thyroid material with 5 per cent sodium hydroxide. Acidification of this solution precipitates the insoluble products. The filtrate is a clear, light yellow solution of the acid-soluble products. If starch is added to this solution and a dilute solution of sodium nitrite is then carefully added, with the formation

of a ring, it is possible to detect the presence of minute traces of iodine if present. When such a test is applied to fresh or desiccated normal thyroid material iodine is not found in the inorganic form. I have found that glands removed at operation from patients with various forms of goiter have inorganic iodides only when Lugol's solution had previously been administered. The iodine was present in the gland either in the inorganic form or it was so loosely combined to the protein that it was broken off with sodium hydroxide. All types of goitrous glands which were removed from patients not receiving Lugol's solution did not contain inorganic iodine or iodine in a form which could be broken off with the alkali.

At the time of my original investigation of the hydrolysis of the proteins of the thyroid with sodium hydroxide, that portion insoluble in acids, following hydrolysis with 5 per cent sodium hydroxide, was designated fraction "A" and the portion soluble in acid, fraction "B." Before the isolation of thyroxine it was considered that all the iodine in "A" occurred in one form and represented the physiologically active, iodine-containing compound of the thyroid. Ingvaldsen and Cameron have fractioned thyroid material in a similar manner and have also assumed that the iodine in the acid-insoluble fraction was present as thyroxine.

Later work in my laboratory, however, has shown that the "A" fraction contains, in addition to thyroxine, iodine compounds which will not bring about physiological changes similar to those following the administration of desiccated thyroid. The percentage of the total iodine which is found in the "A" fraction does not vary markedly with the seasonal change, and it constitutes between 35 and 45 per cent of the total iodine.

Although it might be assumed that alkaline hydrolysis is too severe to use in an attempt to determine the form of iodine present in the thyroid proteins, it is significant that hydrolysis with 10 per cent sulfuric acid, as originally suggested by Baumann, also fractions the iodine-containing compounds into two groups, those soluble in sulfuric acid and those insoluble. With this type of hydrolysis from two-thirds to three-fourths of the total iodine is found to be soluble in the presence of sulfuric acid. This is about the same proportion of the total iodine soluble in acid after alkaline hydrolysis. The solubility in acid is not due to the presence of a high concentration of acid, because neutralization with alkali followed by subsequent small additions of acid may not produce even turbidity in the solution.

The percentage of the total iodine which is soluble in acid after alkaline hydrolysis is remarkably constant. In fresh, normal glands variations in the percentage of sodium or barium hydroxide produce almost no effect. It has been found, however, that in glands removed from patients with exophthalmic goiter, the percentage of the total iodine which was soluble in acid was high and approached 90 per cent (Wilson and Kendall). This may be due to the small amount of iodine compounds present. The slight increase in the amount of acid-insoluble products which were dissolved would produce a correspondingly large increase in the total percentage which would be soluble.

Since Nürnberg,[372] Kendall,[266] Weir, and others have shown that alkaline hydrolysis of the thyroid proteins does not result in the formation of any alkali iodides, evidence is conclusive that some iodine compound is present in the thyroid proteins which is soluble in acids after alkaline hydrolysis. This compound cannot be thyroxine. It may be a decomposition product of a derivative of oxyphenyltyrosine or it may be di-iodotyrosine.

Ingvaldsen and Cameron attempted to prove the presence of di-iodotyrosine in the thyroid by hydrolysis with barium hydroxide. They showed that di-iodotyrosine gives a color reaction with nitrous acid and ammonia similar to that given with thyroxine. This test was positive in the filtrate after acidification of the barium hydroxide solution. It was impossible to crystallize or separate di-iodotyrosine in pure form, but it was shown that di-iodotyrosine added to similar solutions of hydrolyzed protein could not be recovered.

The chemical properties of the acid-insoluble iodine-containing compounds are somewhat revealed by the nature of the products separated at each step in the method for its isolation (Kendall and Simonsen). Following the initial hydrolysis of the thyroid proteins, there is a second treatment with 5 per cent sodium hydroxide. This does not destroy thyroxine but it results in rendering acid-soluble a certain percentage (13) of the total iodine which was acid-insoluble following the first hydrolysis.

Another fractionation of the iodine compounds is obtained by the addition of barium hydroxide to the second sodium hydroxide solution: a variable percentage of the iodine is precipitated by the barium. This fraction is found to vary not only with the season but with the status of the thyroid gland.

One group of thyroid glands from sheep that had been fed potassium iodide contained almost no iodine compounds which were pre-

cipitated by barium at this stage; however, thyroid glands removed from animals in January may show a high percentage of the iodine compounds precipitated by barium hydroxide even in the presence of 5 per cent sodium hydroxide.

The percentage of iodine compounds which are stable and acid-insoluble following these three steps is found to be approximately a constant value (20) of the total iodine: not more than about 10 per cent of the total iodine can be separated from this fraction as thyroxine. Treatment with concentrated barium hydroxide solution still further fractionates the iodine compounds, destroying some and separating thyroxine as the insoluble barium salt.

Thyroxine is separated from that portion of the total iodine which is insoluble in a concentrated barium hydroxide solution by its insolubility in alcohol in the presence of acetic acid. All of the iodine-containing compounds insoluble in concentrated barium hydroxide solution are not thyroxine. The mother liquor following the crystallization of thyroxine may be retreated with barium hydroxide. This gives a barium-insoluble salt of iodine-containing compounds which are stable in strong alkali, but which cannot be crystallized from an alcoholic solution as thyroxine. (See Fig. 9, on page 56.)

I have noted that the iodine-containing compounds which are not thyroxine are broken down with liberation of inorganic iodides, after they have been precipitated with acid and exposed to molecular oxygen, although the iodine is stable to the initial hydrolysis with 5 per cent sodium hydroxide.

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TABLE 4. *Iodine in Hydrolyzed Fractions of Thyroid Material (Kendall and Simonsen).*

Number	Date	Weight in Kilograms	Source of Material *	Total Iodine in Grams	Iodine in Acid Insoluble Fraction *A*	Iodine in Fraction *A* Soluble in Dilute Barium Hydroxide *C*	Iodine in Fraction *C* Insoluble in Acid	Weight of Fraction Insoluble in 40 Per Cent Barium Hydroxide *D* Grams	Weight of Fraction Soluble in 40 Per Cent Barium Hydroxide *E*	Iodine in Fraction *D*	Iodine in Fraction *E*	Weight of Thyroxine
1.	11–26–26	45	H	20.7	8.0	3.5						
2.	12– 3–26	45	B	25.5	9.7	3.1						
3.	12–16–26	45	S	14.4	3.8	1.1						
4.	2– 1–27	1.36	S	2.9		1.61		1.150	0.350	0.443	0.378	0.450
5.	2– 7–27	45	H	15.61	5.7	2.6						0.330
6.	3– 4–27	1	DS	5.6	3.06	1.8				0.600		0.440
7.	2– 9–27	1	DS	5.0	2.5	0.865						
8.	2– 9–27	8.86	S	4.0	1.9	0.636						
9.	2–19–27	2.72	S	0.778		0.171						
10.	3–10–27	45	H	15.5	5.6			4.3	0.900		0.291	0.
11.	3– 2–27	1.2	DH	2.9	1.38	0.535		2.1	0.700		0.082	0.
12.	3–22–27	7.70	S	1.4	0.744	0.298						
13.	3–22–27	2.26	S	0.184	0.059	0.045						
14.	4– 4–27	1	DS	5.9	2.4	1.6		2.05	0.550	0.762	0.183	0.
15.	4– 6–27	45	H	17.7	7.7	2.8		4.9	1.55	1.44	0.436	0.700
16.	4– 8–27	0.325	H	0.402	0.122	0.092						
17.	4–30–27	45	H	21.77	9.29		4.93	8.5	1.5	3.04	0.371	2.21
18.	5–23–27	45	H	29.92	9.45		5.29	11.19	2.48	3.64	0.615	2.85
19.	6– 2–27	45	H	28.88	11.95		6.02	9.65	1.93	3.43	0.433	2.41
20.	6–19–27	45	H	30.0	15.41		7.59	14.73	2.16	4.19		2.83
21.	7– 6–27	1.35	H	0.502	0.226							
22.	7–12–27	45	H	32.05	12.10	10.20	6.92	22.48	3.45	4.71	0.886	3.43
23.	7–13–27	0.963	H	0.921	0.254							
24.	7–13–27	0.771	H	0.343	0.169							
25.	7–14–27	45	H	35.04	17.73	13.87	7.93	15.02	3.52	4.43	0.661	2.37
26.	7–25–27	45	H	36.63	12.39	11.41	7.87	13.40	1.79	5.25	0.398	3.64

27.	8– 1–27	45	H	38.25	14.87	12.77	7.51	18.20	2.55	4.46	0.544	2.89
28.	8–29–27	45	H	37.59	14.01	11.30	6.97	14.17	2.92	3.92	0.713	3.45
29.	8–31–27	45	H	34.17	15.18	14.81	5.85	11.70	3.08	3.79	0.711	2.72
30.	9– 7–27	2.7	H	3.99	1.90							
31.	9–21–27	2.7	H	3.38	1.57	1.30	1.07	2.8	0.250	0.285	0.102	0.
32.	9–21–27	1.0	DS	1.76	1.12	0.991	0.766	0.230	0.590	0.065	0.092	0.
33.	9–28–27	1.64	S	0.364	0.166	0.095	0.031					
34.	9–28–27	1.11	S	0.255	0.102	0.088	0.035					
35.	9–28–27	1.86	S	0.233	0.099	0.052	0.021					
36.	10– 4–27	45	H	28.69	10.78	9.91	5.71	13.2	3.42	4.24	0.774	2.21
37.	10–10–27	1.0	DS	1.73	1.04	0.887	0.482	1.47	0.500	0.201	0.054	0.
38.	10–14–27	0.850	S	0.292	0.112							
39.	10–14–27	1.07	S	0.218	0.084							
40.	11– 2–27	45	H	34.45	10.72	8.97	6.06	12.59	3.30	4.22	0.729	2.25
41.	12– 8–27	45	H	31.40	12.03	10.71	6.50	12.47	3.01	5.24	0.742	2.56
42.	1– 3–28	45	H	22.23	9.90	8.77	4.66	8.51	2.32	2.83	0.780	1.70
43.	2– 7–28	45	H	16.71	7.03	6.05	3.31	6.55	2.66	1.78	0.584	0.85
44.	3– 2–28	45	H	14.71	6.67	5.41	3.44	6.11	1.55	2.26	0.329	1.53
45.	5–11–28	45	H	19.90	8.26	5.80	3.98	7.5	2.55	2.43	0.615	1.20
46.	6– 2–28	45	H	25.94	10.95	10.32	6.97	12.29	4.05	3.99	0.800	2.00
47.	7– 6–28	45	H	27.29	8.84	7.26	5.10	14.2	3.98	4.38	0.548	2.15
48.	8–11–28	45	H	36.99	17.30	13.21						

* B—Beef; D—Desiccated; H—Hog; S—Sheep.

TABLE 5. *Percentage of Total Iodine in the Hydrolyzed Fractions Given in Table 4 (Kendall and Simonsen).*

Number	Per Cent of Total Iodine Insoluble in Acid after Hydrolysis *A*	Per Cent of Total Iodine in *A* after Addition of Dilute Barium Hydroxide *C*	Per Cent of Total Iodine in *C* Insoluble in Acid	Per Cent of Total Iodine Insoluble in 40 Per Cent Barium Hydroxide *D*	Per Cent of Total Iodine Soluble in 40 Per Cent Barium Hydroxide *E*	Per Cent of Total Iodine in Form of Thyroxine
1.	39	17				
2.	38	12				
3.	26	7.6				
4.		56		15	13	10
5.	37	17				1.4
6.	55	32		11		5.2
7.	50	17				
8.	47	16				
9.		22				
10.	36				1.9	0.
11.	48	19			2.8	0.
12.	53	21				
13.	32	24				
14.	41	27		13	3.1	0.
15.	43	16		8.1	2.4	2.6
16.	30	23				
17.	43		23	14	1.7	6.7
18.	32		18	12	2.0	6.3
19.	41		21	12	1.4	5.5
20.	51		25	14		6.1
21.	45					
22.	38	32	22	15	2.7	7.0
23.	28					
24.	49					
25.	51	40	23	13	1.8	4.5
26.	34	31	21	14	1.0	6.6
27.	39	33	19	12	1.4	5.0
28.	37	30	19	10	1.8	6.1
29.	44	43	17	11	2.0	5.3
30.	48					
31.	46	38	32	8.4	3.0	0.
32.	64	56	44	3.6	5.2	0.
33.	46	26	8.5			
34.	40	35	14			
35.	42	22	9.0			
36.	38	35	20	15	2.6	5.1
37.	60	51	28	12	3.1	0.
38.	38					
39.	39					
40.	31	26	18	12	2.1	4.3
41.	38	34	21	17	2.3	5.4
42.	45	39	21	13	3.5	5.0
43.	43	36	20	11	3.4	3.3
44.	45	37	23	15	2.2	6.9
45.	42	29	20	12	3.1	3.9
46.	42	40	27	15	3.1	5.1
47.	32	29	19	16	2.0	5.3
48.	52	36				

The results which have just been described are obtained by alkaline hydrolysis of fresh thyroid glands. When certain samples of desiccated thyroid are treated in a similar way, the total iodine is divided into approximately the same percentage of acid soluble and insoluble products but the percentage of total iodine which can be separated as thyroxine may approach zero. This observation suggested the possibility of a change in the chemical properties of thyroxine during and after desiccation and removal of fat. In order to test the stability of the iodine compounds in the thyroid, Kendall and Simonsen bubbled air through 45 kg. of finely-ground fresh hog thyroid glands suspended in a water solution for forty-eight hours. The temperature was approximately 25°. At the end of this time sodium hydroxide was added and the usual hydrolysis was carried out. There was a small but significant increase in the percentage of the acid soluble iodine-containing compounds and iodide was found in the solution. Furthermore, there was a marked difference in the distribution of the iodine-containing compounds which were stable to the second sodium hydroxide treatment, and which were soluble in the presence of barium. Finally, there was a significant decrease in the percentage of the total iodine which could be separated as thyroxine. A control experiment carried out with 45 kg. of the same sample of hog thyroid without treatment and another with an equal amount treated with carbon dioxide are given in Table 6. The variations produced by aerating the solution are much greater than those formed in duplicate samples of non-aerated glands.

Since the thyroid proteins suspended in water are decomposed and altered and since there are marked variations in the distribution of the iodine-containing compounds, it would appear probable that changes may occur during the preparation of desiccated thyroid, which would bring about alteration of thyroxine and the other iodine compounds contained in desiccated thyroid. For the assay of thyroid material for thyroxine by chemical means, only fresh thyroid material should be used. It may, however, be possible to desiccate thyroid under conditions which minimize or prevent alteration. That this is possible is indicated by the fact that Harington was able to treat desiccated thyroid and secure a large percentage of the total iodine in the form of thyroxine.

From the investigations of the compounds containing iodine in the thyroid there seems to be no escape from the conclusion that the iodine is present in more than one form of combination, and there are sufficient chemical data at hand to make certain that all of the iodine does not occur as thyroxine. Since treatment of some samples of thyroid

TABLE 6. *Changes in the Distribution of the Iodine Compounds Produced by Allowing the Material to Stand 72 Hours Suspended in Water Before Hydrolysis with Alkali (Kendall and Simonsen).*

Number	Date	Weight in Kilograms	Source of Material	Total Iodine	Iodine in Acid-Insoluble Fraction *A*	Iodine in Fraction *A* Soluble in Dilute Barium Hydroxide *C*	Iodine in Fraction *C* Insoluble in Acid	Weight of Fraction Insoluble in 40 Per Cent Barium Hydroxide *D*	Weight of Fraction Soluble in 40 Per Cent Barium Hydroxide *E*	Iodine in Fraction *D*	Iodine in Fraction *E*	Weight of Thyroxine
				Grams								
1	3–28–28	45	H	18.36	5.38	3.83	2.18	3.55	1.10	1.29	.268	.635
2	4– 4–28	45	H	18.86	7.38	5.39	3.08	8.40	2.45	1.73	.701	1.15
3	4– 7–28	45	H	16.11	5.44	3.74	2.18	3.25	2.25	1.14	.591	.400

Number	Date	Weight in Kilograms	Source of Material	Total Iodine in Grams	Per Cent of Total Iodine Insoluble in Acid after Hydrolysis *A*	Per Cent of Total Iodine in *A* after Addition of Dilute Barium Hydroxide *C*	Per Cent of Total Iodine in *C* Insoluble in Acid	Per Cent of Total Iodine Insoluble in 40 Per Cent Barium Hydroxide	Per Cent of Total Iodine Soluble in 40 Per Cent Barium Hydroxide	Per Cent of Total Iodine in Form of Thyroxine
1	3–28–28	45	H	18.36	29	21	12	7.0	1.4	2.3
2	4– 4–28	45	H	18.86	39	29	16	9.1	3.7	3.8
3	4– 7–28	45	H	16.11	34	23	14	7.0	3.6	1.6

H: hog thyroid.

No. 1. 45 kg. hydrolyzed with sodium hydroxide after standing suspended in tap water for 72 hours. Air was passed through the solution continuously.
No. 2. 45 kg. hydrolyzed with sodium hydroxide immediately.
No. 3. 45 kg. hydrolyzed with sodium hydroxide after standing suspended in tap water for 72 hours. Carbon dioxide was passed through the solution continuously.

material with barium or sodium hydroxide indicates that the thyroxine content approaches zero, it is difficult to escape the conclusion that from 15 to 20 per cent is a maximum, and that usually not more than 7 per cent of the total iodine is in the form of thyroxine.

If the weight of thyroxine which is actually separated in crystalline form is accepted as an approximation of the total amount present, some samples of desiccated thyroid which contain a normal amount of iodine, have none of this iodine in the form of thyroxine. In Harington's [200] publication concerning the isolation of thyroxine, he stated that, by the method described, thyroxine can be isolated from desiccated thyroid in a yield of about 0.125 per cent of the dried gland. This statement is misleading, since the particular sample of desiccated thyroid treated

by Harington when this yield was obtained possessed unusual properties. Samples of American desiccated thyroid which I have investigated thus far have not contained thyroxine in this amount, and none of five other samples of desiccated thyroid obtained from England contained this amount, when assayed by Harington's method.

One sample of material was prepared especially for this determination. Desiccated thyroid prepared from sheep which were grown in Yorkshire, England, was secured through the courtesy of the British Drug Houses, and, although these glands were removed from the animals in the month of June, the iodine content was unusually low, 0.19 per cent, and thyroxine could not be isolated.

The highest percentage of the total iodine which has been separated in the form of thyroxine is 14 per cent (Harington).[200] This yield was 0.125 per cent of the weight of the dry gland. The highest percentage of dry fat-free thyroid material separated as thyroxine was obtained in my laboratory from the thyroid glands of sheep which had been fed iodine for nine months; the total iodine present was over 1 per cent and the yield of crystalline thyroxine was 0.400 per cent of the dried weight of the gland.

DOES IODOTHYRIN CONTAIN THYROXINE?

An interesting question in regard to the chemical nature of the iodine-containing products which have been separated from the thyroid gland concerns the presence of thyroxine in iodothyrin. Samples of iodothyrin have been prepared by many investigators; these have been shown to possess the typical physiological activity of thyroid material. Other preparations have been almost physiologically inert, although iodine was present. Since thyroxine is stable to boiling sulfuric acid, it seemed probable that preliminary treatment of the thyroid material with 10 per cent sulfuric acid would break down the proteins and yield a fraction which could be further treated with barium hydroxide. It seemed probable that this preliminary treatment would increase the yield of crystalline thyroxine which could be separated from thyroid material.

In order to decide this question, English desiccated thyroid containing 0.54 per cent of iodine was hydrolyzed with 10 per cent sulfuric acid for five hours (Kendall and Simonsen). The iodothyrin was separated according to the method of Baumann, and it was shown to be physiologically active through administration to thyroidectomized swine; marked improvement and increase in weight followed. It was found impossible to separate any crystalline thyroxine from this sample of

desiccated thyroid after alkaline hydrolysis either with sodium or barium hydroxide and when the iodothyrin which had been separated was treated with 40 per cent barium hydroxide, it was not possible to separate crystalline thyroxine from the barium-insoluble fraction.

It is possible, after mixing crystalline thyroxine with gelatine and after hydrolysis with sodium hydroxide to recover the thyroxine in good yield. Five hundred mg. of pure, crystalline thyroxine was dissolved in 500 cc. of 5 per cent sodium hydroxide and to this solution 25 grams of gelatine was added. The solution was heated for twenty-four hours. Forty-four mg. of organically-bound iodine was in the acid-soluble fraction. The acid-insoluble material was dissolved in alkaline alcohol to which acetic acid was added; 310 mg. of crystalline thyroxine was recovered. This result clearly shows that thyroxine is not destroyed by the action of sodium hydroxide in the presence of gelatine. We may therefore conclude that thyroxine was not present in the iodothyrin and that the derivative of thyroxine which was present is stable to acid hydrolysis but not to the action of alkaline hydrolysis.

After it had been shown that pure, crystalline thyroxine which had been mixed with gelatine could be almost quantitatively recovered after treatment with 5 per cent sodium hydroxide it seemed probable that the inability to separate thyroxine from iodothyrin was due to the effect of acid on thyroxine in the presence of protein material. This possibility was investigated by the hydrolysis of 25 grams of gelatine in 500 cc. of 10 per cent sulfuric acid to which 500 mg. of crystalline thyroxine had been added.

The solution was boiled for five hours; it was then cooled and filtered. Thirty-eight mg. of iodine was in the acid-soluble fraction although inorganic iodides were not present. The acid-insoluble precipitate weighed 445 mg. and from this 350 mg. of crystalline thyroxine was separated. The recovery of such a high percentage of the total thyroxine which had been added to the gelatine shows conclusively that small amounts of thyroxine can be isolated after hydrolysis of protein with acid; this is still further evidence that the iodothyrin did not contain appreciable amounts of thyroxine.

Although this sample of iodothyrin prepared from desiccated thyroid had marked thyroid activity but did not contain any thyroxine, other samples of iodothyrin prepared from fresh glands may contain thyroxine which can be separated in crystalline form.

Thyroxine was also heated in 10 per cent sulfuric acid with alanine and with a mixture of alanine and glucose. In neither of these experi-

ments was the thyroxine appreciably decomposed, and it was recovered unchanged for the most part. Similar experiments were also carried out with 25 grams of gum acacia in 500 cc. of 5 per cent sodium hydroxide to which 500 mg. of thyroxine was added. The thyroxine was recovered in about the same yield as from a gelatine solution. The same amount of thyroxine added to 25 grams of gum acacia in 500 cc. of 10 per cent sulfuric acid showed that this solution did not destroy the thyroxine, although some of the material appeared to be carried down in the fraction which is insoluble in neutral alcohol.

Iodine was not broken off by either the acid or the alkaline treatment and thyroxine was but slowly altered when boiled in a solution of gelatine, amino acids, or gum acacia with 5 per cent sodium hydroxide or with 10 per cent sulfuric acid. This stability of thyroxine is in striking contrast to the high percentage of the total iodine which is soluble in acid after hydrolysis of thyroid material. No treatment has been found by which the products soluble in acid subsequently become acid-insoluble and it appears certain that this fraction of the iodine-containing compounds cannot be derived from thyroxine by any method of degradation of thyroxine itself. The ether linkage between the two benzene rings of thyroxine requires drastic treatment before it can be broken, and it has been repeatedly shown that 5 per cent sodium hydroxide cannot decompose the thyroxine molecule with liberation of iodine. Quite apart from any physiological interpretation, it would appear that thyroxine must represent an intermediate form in the elaboration of the physiologically active compound, which is probably derived directly from the thyroxine.

From a chemical viewpoint variations of the iodine content in the thyroid gland amounting to 300 per cent are significant. The sudden decrease in the amount of iodine in the thyroid gland in the winter months and the equally sudden increase in the spring months cannot be related to iodine in the food alone.

The determination of the fluctuations of the iodine in the gland furnishes a much more powerful tool for the investigation of this problem than many of the other avenues of approach, for example, the degree of hyperplasia, or the changes in density of the colloid material as determined by histological section.

The influence of thyroidectomy does not give data which can be interpreted quantitatively. It is a remarkable fact that the entire thyroid gland may be removed from an adult animal such as the swine or sheep without producing any appreciable effect in the appearance or the habits

of the animal. It is equally surprising to find that in a normal adult animal the thyroid is actually responding to many factors other than the iodine content of the food which influence it so profoundly that the iodine content of this organ varies between wide limits.

Two significant observations have been made in my laboratory concerning the solubility of the iodine-containing compounds of the thyroid: (1) Haines and Mulholland [528] found that a large percentage of the total iodine present in fresh thyroid tissue diffuses out of the glandular structure and is found soluble in 10 per cent formaldehyde; as much as 50 per cent or more of the iodine in some glands is found present in the formaldehyde solution used to preserve the cellular structure of the gland; (2) the iodine compounds in thyroid material which has been dried cannot be redissolved with physiological salt or with a phosphate buffer, pH 7.4; although the desiccated thyroid remained in suspension in these solutions for many hours, subsequent removal of the undissolved proteins and determination of iodine in the solution revealed the surprising fact that the iodine-containing compounds are practically quantitatively insoluble in physiological salt or in phosphate buffer, pH 7.4, after the proteins have been dried.

Many pharmacological and physiological investigations of the thyroid gland have been reported in which aqueous or saline extracts of desiccated thyroid have been employed. No determinations of the iodine content of the solutions were given by the authors and it is doubtful whether any of the iodine-containing compounds of the thyroid were contained in these extracts.

These results further emphasize the fact that thyroxine and derivatives of this compound in the gland do not exist in the free condition, but are intimately and firmly bound to the proteins of the thyroid.

SUMMARY

Inorganic iodine is not present in the normal thyroid gland. Sodium hydroxide does not break off iodine from any compound in the normal thyroid gland. Inorganic iodides are found after hydrolysis with sodium hydroxide, if iodide or Lugol's solution has been recently administered. After hydrolysis with alkali the organic compounds containing iodine may be divided into acid-soluble and acid-insoluble fractions. All of the thyroxine is contained in the acid-insoluble fraction, but iodine-containing compounds other than thyroxine are also present. There are variations in the relative amounts of the iodine-containing compounds of the thyroid gland. Di-iodotyrosine may be present in the gland but

up to the present it has not been isolated in crystalline form. All of the iodine in the gland cannot be present in the same form of combination.

Acid hydrolysis divides the iodine-containing compounds into fractions soluble and insoluble in acid. The distribution of iodine in these fractions is approximately the same as that obtained by alkaline hydrolysis. The iodine in thyroxine is not more than from 0 to 15 per cent of the total iodine in the gland. The highest percentage of the total iodine which has been separated as thyroxine is 14 and the highest percentage of the thyroid material which has been separated as thyroxine is 0.4.

Iodothyrin, which possesses marked physiological activity, may not contain thyroxine which can be isolated in crystalline form. Since thyroxine can be recovered after it has been added to protein or gum acacia and since it is stable to alkaline or acid hydrolysis, it seems probable that thyroxine can exist in a physiologically active form not stable to alkali. Chemical examination of the thyroid gland suggests that thyroxine is an intermediate form of the physiologically active compound. Although the iodine compounds in the thyroid gland are easily extracted by physiological salt solution from fresh thyroid material, after the proteins have been desiccated only traces of the iodine compounds are extracted with physiological salt or with phosphate buffer (pH 7.4).

Chapter 7

The Influence of the Thyroid on Hypothyroidism, Nitrogen, Phosphorus and Calcium Metabolism and on the Distribution of Water

THE EFFECT OF THYROID MATERIAL IN HYPOTHYROIDISM

It was but natural that the physiological and chemical investigation of the thyroid gland should follow the striking clinical results obtained by administration of this material, but before progress could be made in this problem it was necessary to find a method for the assay of thyroid activity. The improvement in the clinical condition of patients

FIG. 10.—Patient with high-grade myxedema before treatment with the acid-insoluble fraction obtained by alkaline hydrolysis of thyroid material.

FIG. 11.—Same patient as shown in Fig. 10 after treatment with the acid-insoluble fraction for a few weeks.

suffering from hypothyroidism was at first the only criterion of the physiological activity of this gland. Quantitative observations could not be made, but the change in the heavy, edematous features in a case of myxedema was among the most striking effects which had been observed in the whole field of medicine, and was convincing evidence of the physiological activity of the products under investigation.

FIG. 12.—Appearance of patient on entering the clinic.

FIG. 13.—Same patient as shown in Fig. 12 after eighteen days' treatment with the acid-insoluble products of alkaline hydrolysis of the thyroid.

FIG. 14.—Appearance of patient on entering the clinic.

FIG. 15.—Same patient as shown in Fig. 14 after eighteen days' treatment with small amounts of the acid-insoluble products of alkaline hydrolysis.

FIG. 16.—Appearance of patient on entering the clinic.

FIG. 17.—Same patient as shown in Fig. 16 after twenty-four days' treatment with the acid-insoluble products of alkaline hydrolysis of the thyroid.

FIG. 18.—Appearance of patient on entering the clinic.

FIG. 19.—Same patient as shown in Fig. 18 after twenty-four days' treatment with the acid-insoluble products of alkaline hydrolysis of the thyroid.

The impotence of potassium iodide or any other iodine compound except the one found in the thyroid gland could be demonstrated with certainty, and this criterion for the activity of thyroid material has continued to be valuable and important. A compound credited to be the physiologically active constituent of the thyroid cannot be regarded as such unless it relieves the condition of hypothyroidism to an extent equal to that produced by administration of thyroid material. This method, however, has been criticized by Pick and Pineles, who suggested that variations in the activity of the thyroid gland of the patient with myxedema could explain the relief following the administration of thyroid products. It now appears certain that this objection is not valid. The work of Plummer on the influence of thyroxine in cases of myxedema has shown that the thyroid gland which has been injured to the extent that frank symptoms of myxedema develop cannot be regenerated.

FIG. 20.—Same patient as shown in Figs. 16, 17, 18 and 19, almost five years later, during which time she had continuously taken the acid-insoluble products of alkaline hydrolysis of the thyroid.

It is possible to increase the activity of the thyroid gland under some conditions, one of which is after partial atrophy due to long-continued inanition. If the basal metabolic rate of such an individual is brought to normal by the administration of thyroxine it is possible for the thyroid gland to resume its normal function; not only may the amount of thyroxine which is required to maintain the normal basal metabolic rate be reduced, but eventually all thyroid medication may be stopped (Plummer and Haines). Plummer has noted this result in many cases of low basal metabolic rate not due to atrophy of the thyroid, and Davidson has reported a dramatic result in a case of malnutrition. This is good evidence that the thyroid gland of the patient has resumed its former activity; this result is not seen in any case of true hypothyroidism caused by complete atrophy of the gland.

In all cases of high-grade myxedema the basal metabolic rate may be brought to normal by the administration of thyroxine, irrespective

FIG. 21.—Patient with nephrosis. (Davidson.)

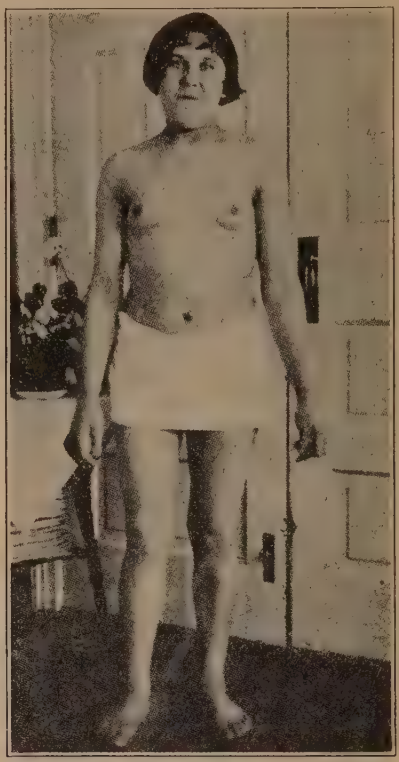


FIG. 22.—Same patient as shown in Fig. 21 after treatment with desiccated thyroid.

of the condition of the thyroid gland, but as soon as it is discontinued the rate again diminishes and eventually returns to the previous level.

RELIEF OF GOITER

A second criterion which was used by early investigators was the influence of thyroid preparations on the size of goiters. Baumann, Roos, Oswald and many others have shown that the administration of thyreoglobulin or iodothyrin reduced the size of some goiters. This method which was used to test for the presence of thyroid material has not proved to be as valuable a criterion as the relief of hypothyroid symptoms. Fresh and desiccated thyroid material, thyreoglobulin, iodothyrin,

and thyroxine will all relieve some types of goiter; it is obvious, however, that a large, degenerating, cystic gland cannot respond to thyroid administration as the simple enlargement of an adolescent goiter does. Thyroiditis and carcinoma of the thyroid are not affected by thyroid medication and, although these conditions are relatively rare, they are difficult to diagnose clinically and they cannot be excluded without histological examination. Physiological activity which is based on the effect of any preparation on goiter may be misleading.

It is also significant that inorganic iodides alone will relieve some forms of goiter, so that this test is not specific for the physiologically active constituent of the thyroid. An extended clinical investigation of the influence of thyroxine and iodides on various types of goiter, carried out by Plummer, has shown that thyroxine will abolish the bruit and reduce the size of some goiters, but that administration of iodides does not have a similar effect. This result indicates that stimulation of the gland is relieved by the administration of thyroxine and that the gland cannot under all conditions utilize iodine for the elaboration of thyroxine.

INFLUENCE ON BODY WEIGHT AND THE NITROGEN BALANCE

In addition to the results obtained by clinical observation of the relief of the symptoms of hypothyroidism and of the size of goiters, the early workers in the field of thyroid activity used only two other criteria in order to study in a quantitative way the effect of thyroid preparations. These were the influence of the thyroid gland on body weight and the increase in the excretion of nitrogen which was associated with the loss in weight. Loss of weight is not a constant accompaniment of the administration of thyroid and it must now be considered as a pathological in distinction to a physiological response. The thyroid-deficient individual does not lose weight on small amounts of thyroid medication; in fact the reverse may occur and rapid bodily development and increase in weight may follow the proper dosage of thyroid material. Loss of weight occurs when excessive amounts of thyroid material are administered, and the correct explanation is undoubtedly associated with the increase in the basal metabolism. When the oxygen consumption and carbon dioxide output are increased from 50 to 75 per cent above normal and the level of food intake is not increased, it follows that the body weight must decrease.

This criterion for active thyroid material has been used but it is obviously not quantitative and the response of different species of

animals to thyroid medication is strikingly different. Pure, crystalline thyroxine administered to experimental dogs, goats and monkeys brought about a prompt and marked loss of body weight: if a small amount of thyroxine was given over a long period of time the loss of weight was carried to an extreme. (See Figs. 23 and 24.)

Fig. 23. Fig. 24.

Fig. 23.—Normal monkey before injection, weight 6.14 kilos.
Fig. 24.—Same monkey as shown in Fig. 23 after seventy-two daily injections of the products of alkaline hydrolysis insoluble in acid which contained 1.5 mg. of iodine. Weight 2.72 kilos.

In man the nervous system may be so affected by the administration of thyroid material that it is impossible to administer enough to induce loss of weight without the production of distressing subjective symptoms.

In 1893, Müller [860] showed that, although in the majority of cases of exophthalmic goiter death did not occur as a direct result of the disease, some patients became critically ill and died after an unusually severe course. In some cases there was great emaciation.

Müller was the first to show by quantitative determination that in exophthalmic goiter there is a marked increase in the nitrogen output; even though the daily intake of nitrogen was raised to 52.59 grams, the output was 57.27 grams. Indeed, it was found impossible to maintain a nitrogen equilibrium on a high protein intake.

It was recognized that exophthalmic goiter was the result of over-

activity of the thyroid gland, and the abnormal breakdown of protein in this condition was highly suggestive that protein metabolism was in some manner influenced by the activity of this gland. The clinical observations and laboratory results obtained by Müller were soon confirmed by other evidence secured by administration of thyroid material to experimental animals and patients with various degrees of hypothyroidism.

In the same year as Müller published his results Mendel gave a sterilized glycerine extract of the thyroid gland to a patient with

TABLE 7. *Progress of Patient Under the Thyroid Gland Treatment (Napier).*

Days	Thyroid Gland Given	Weight	Urine		
			Volume Cubic Centimeters	Specific Gravity	Urea: Grams in 24 Hours
1	.....	...	1036	....	..
2	Thyr.	198	1540	1.015	22
3	.....	...	1148	1.018	30
4	Thyr.	...	2128	1.015	35
5	.....	...	1568	....	..
6	.....	...	1568	....	..
7	Thyr.	...	1904	....	..
8	.....	...	1288	....	..
9	.....	193	2632	....	..
10	.....	...	1428	....	..
11	Thyr.	...	1428	....	..
12	.....	...	1176	....	..
13	Thyr.	...	1904	1.016	41
14	.....	...	1680	....	..
15	.....	187	1904	....	..
16	Thyr.	...	1456	1.019	45
17	.....	...	1680	....	..
18	.....	...	1680	....	..
19	Thyr.	...	1232	1.021	41
20	.....	...	1428	....	..
21	Thyr.	...	1680	1.019	40
22	.....	183½	1568	....	..
23	Thyr.	...	1288	1.022	37
24	.....	...	1176	....	..
25	.....	...	1260	1.021	36
26	.....	...	1176	....	..
27	Thyr.	...	1176	....	..
28	.....	...	1400	1.020	37
29	.....	178½	1764	1.021	55
30	.....	...	1260	....	..
31	.....	...	1176	1.021	31
32	.....	...	1316	....	..
33	.....	...	1456	1.020	42
34	Thyr.	...	1008	1.021	28
35	.....	...	1092	....	..
36	.....	176	1344	....	..
37	Thyr.	...	1232	1.019	32
38	.....	173	....	....	..

TABLE 8. *The Influence of Desiccated Thyroid on the Excretion of Nitrogen of a Dog (Roos).*[430]

Days	Urine Volume in 24 Hours Cubic Centimeters	Specific Gravity	Nitrogen Grams	Body Weight Grams	
1	500	1.012	3.220	8500	
2	490	1.014	3.052		
3	510	1.014	3.088	8500	
4	560	1.012	2.940		
5	550	1.013	3.060	8500	
6	480	1.013	2.839		
7	600	1.012	3.654	8520	
8	410	1.016	2.706		
9	430	1.016	2.754	8420	
10	500	1.015	3.080		3 grams desiccated thyroid.
11	530	1.015	3.598	8270	
12	510	1.019	3.944		
13	560	1.019	4.312	8000	
14	500	1.018	3.920		
15	490	1.019	4.030	7950	
16	420	1.020	3.586		
17	800	1.020	3.22		
18			3.22	7950	
19	490	1.016	3.052	7950	
20	430	1.020	3.401		
21	270	1.022	2.513	7970	
22	570	1.014	3.371		
23	400	1.021	3.36	7900	
24	470	1.019	3.364		
25	480	1.017	3.44	7850	
26	480	1.016	3.44		6 grams desiccated thyroid.
27	600	1.015	4.221	7630	
28	610	1.019	4.334		
29	350	1.027	4.128	7540	
30	480	1.019	4.015		
31	480	1.019	4.032	7530	
32	450	1.017	3.181		
33	480	1.020	3.544	7600	
34	180	1.027	1.732		
35	280	1.018	2.058		
36	290	1.022	2.649		
37	390	1.016	2.702	7480	
38	420	1.017	2.691		
39	520	1.017	3.658	7485	
40	300	1.019	2.593		

myxedema, and was the first to point out that administration of thyroid material caused an increase in the urea output. The daily urea excretion was increased from 14.3 grams to 36.4 grams.

Napier showed that administration of fresh thyroid gland of sheep produced diuresis and a large increase in the excretion of urea.

Vermehren showed an increase of from 200 to 300 per cent in the nitrogen output of a patient with myxedema after treatment with fresh and cooked thyroid glands.

At the same time, Ord and White, and Roos,[430] Bleibtreu and Wendelstadt, and Dennig [122, 123] showed that the administration of desiccated thyroid caused diuresis and increased the nitrogen output.

Dinkler treated three patients with desiccated thyroid and determined both the intake and output of nitrogen. There was a marked increase in the nitrogen output following the administration of thyroid. The many observations in clinical medicine which were made before Baumann reported his significant contribution, closely linked the activity of the thyroid gland to protein metabolism.

As soon as iodothyrin became available Roos [431] showed that this substance brought about a change in body weight and an increase in nitrogen output which was similar to the effect brought about by desiccated thyroid.

TABLE 9. *The Influence of Thyreo-antitoxin and Iodothyrin on the Excretion of Nitrogen (Roos).*[431]

Days	Urine Volume Cubic Centimeters	Specific Gravity	Nitrogen Grams	Body Weight Grams	
1	345	1.017	2.535	6290	
2	275	1.016	2.483	6350	
3, 4	600	1.016	2.562	6400	
5	280	1.016	2.332	6440	
6	260	1.016	2.787	6490	
7	260	1.014	2.402	6470	Thyreo-antitoxin from 76 grams of gland.
8	340	1.016	2.415	6520	
9	280	1.012	2.097	6500	
10	280	1.011	1.911	6450	
11	300	1.018	2.268	6520	
12	310	1.016	2.844	6520	Thyreo-antitoxin from 85 grams of gland.
13	300	1.020	2.803	6520	
14, 15	660	1.018	2.234	6570	
16	280	1.014	2.214	6590	
17	260	1.018	2.329	6550	Iodothyrin from 59 grams of gland.
18	430	1.012	3.687	6470	
19	355	1.017	3.311	6370	
20	280	1.022	3.283	6320	
21, 22	340	1.021	2.046	6400	
23	240	1.021	2.612	6490	

A few years later Roos [434] made the important observation that the activity of thyroid material when measured by the increase in nitrogen output was proportional to its iodine content.

TABLE 10. *The Influence of Variations in the Iodine Content of Thyroid Material on the Excretion of Nitrogen (Roos).*[434]

Days	Urine Volume Cubic Centimeters	Specific Gravity	Nitrogen Content Grams	Body Weight Grams	
1	590	1.023	9.43	13780	
2	660	1.021	9.24	13800	
3	710	1.020	9.46	13910	
4	730	1.020	9.45	13930	5 grams of dog thyroid, iodine-free.
5	650	1.021	9.56	13900	
6	660	1.022	9.70	13980	
7	550	1.022	8.56	14140	5 grams of dog thyroid, iodine-free.
8	590	1.024	9.60	14120	
9	650	1.021	9.05	14100	
10	630	1.023	9.15	14170	
11	640	1.021	9.00	14080	
12	...	....	...	14190	
13	...	....	...	14210	
14	630	1.023	9.30	14260	
15	660	1.022	9.20	14280	
16				14320	
17	570	1.024	9.35	14390	5 grams of dog thyroid containing 17.5 mg. of iodine.
18	660	1.023	10.25	14300	
19	650	1.024	9.91	14220	
20	570	1.025	9.26	14300	
21	580	1.024	8.93	14360	
22	550	1.024	9.18	14550	
23	...	....	...	14620	

The dog was maintained throughout on a diet of 350 grams of dog biscuit, 500 cubic centimeters of milk and 500 cubic centimeters of water.

Fonio gave thyroid material to a patient with myxedema and caused an increase in nitrogen excretion which was accompanied by diuresis and loss of weight. The effect on the nitrogen output was proportional to the iodine content of the material administered. Lanz showed that there was an increase in the nitrogen output only if the gland contained iodine.

During the investigation of the effect of alkali on thyroid material I showed that the acid-insoluble products following alkaline hydrolysis brought about the typical increase in nitrogen excretion observed after administration of the entire thyroid gland. The physiological activity of the hydrolyzed products was determined throughout the various stages of the purification and were finally shown to be brought about by thyroxine itself.

These observations brought to a close the investigation of the relation between nitrogen metabolism, the activity of the thyroid gland and the iodine-containing compound in the gland. The typical effect

TABLE 11. *The Influence of Variations in the Iodine Content of Thyroid Material on the Excretion of Nitrogen (Roos).*[434]

Days	Urine Volume Cubic Centimeters	Specific Gravity	Nitrogen Content Grams	Body Weight Grams	
1	640	1.023	9.43	12010	
2	660	1.023	9.74	12130	
3	720	1.020	9.09	12020	
4	660	1.021	8.77	12050	
5	640	1.021	9.15	12100	5 grams of thyroid gland from child, containing 1.25 mg. iodine.
6	740	1.021	10.25	12120	
7	680	1.020	8.52	12160	
8	705	1.021	9.69	12250	
9				12300	
10	650	1.022	9.28	12450	
11				12550	
12	610	1.022	9.39	12650	5 grams of thyroid gland from child, containing 9 mg. iodine.
13	770	1.020	10.42	12500	
14	685	1.021	10.11	12600	
15	615	1.025	9.60	12480	
16	460	1.024	7.51	12700	
17	620	1.022	9.09	12730	
18	690	1.020	9.22	12820	
19	660	1.020	9.01	12850	
20				12870	
21	680	1.023	9.47	12970	
22	670	1.021	8.60	13040	5 grams of thyroid gland from child, containing 1.25 mg. iodine.
23	730	1.021	10.24	13030	
24	620	1.023	9.33	13110	
25	625	1.023	9.42	13020	
26	660	1.023	9.58	13040	
27	625	1.022	9.53	13070	5 grams of thyroid gland from child, containing 9 mg. iodine.
28	770	1.021	10.78	12930	
29	780	1.021	10.45	12900	
30	545	1.022	8.76	13030	
31	730	1.023	10.85	13050	
32	540	1.023	9.05	13070	
33	590	1,023	8.43	13270	

of fresh or desiccated thyroid material on nitrogen metabolism was produced by pure, crystalline thyroxine alone.

NITROGEN RETENTION

Although thyroxine and desiccated thyroid bring about a great increase in nitrogen elimination when given in sufficient amount, the opposite effect may follow in some conditions.

Hewitt has shown that a small amount of thyroid material admin-

istered to a rat caused retention of nitrogen. Janney and Henderson showed that administration of small amounts of thyroid material brought about retention of nitrogen in a child with hypothyroidism. These results emphasized the anabolic function of the thyroid and showed that whether nitrogen was retained or excreted depended on the amount given and the condition of the patient.

Courvoisier found that iodothyrin produced an increase in the nitrogen output, but that the nucleoprotein from the thyroid brought about nitrogen retention. The production of actual nitrogen retention which is due to the nucleoprotein does not seem probable. There is no question that a proper dose of thyroxine will induce growth in a young animal with hypothyroidism. Whether or not a positive nitrogen balance is also present depends on the caloric requirements and the type and amount of food intake.

THE INFLUENCE OF THE THYROID GLAND ON PROTEIN, FAT AND CARBOHYDRATE METABOLISM

Reviews of the experimental work in relation to thyroid activity and total metabolism have been published by Magnus-Levy,[333] Falta, Du Bois, and Grafe.[179] Definite results have been obtained which have shown that a positive nitrogen balance is maintained in hypothyroidism in man with amounts of protein intake insufficient for a normal person. Von Bergmann observed that in myxedema moderate undernutrition of short duration did not result in a negative nitrogen balance, and he suggested that in a hypothyroid status the nitrogen requirements are particularly low.

In contrast to this result the work of many observers has shown that protein breakdown is increased in hyperthyroidism although it may not be proportional to the severity of the disease or the increase in the basal metabolic rate. There is a negative nitrogen balance with a diet containing a protein and caloric content which is sufficient to maintain metabolic equilibrium in a normal subject. In some cases the increase in protein breakdown was observed even when both the protein and caloric value were raised above normal.

This observation and the fact that administration of active thyroid material always brought about, at least at first, an increase in nitrogen elimination suggested that protein metabolism was controlled by the action of the thyroid gland, and it seemed probable that the protein material in the body was directly and specifically acted on by the active agent of the thyroid. Thyroid material was considered toxic to the

animal organism because of its direct action on protein. This viewpoint was held by many investigators and is well illustrated by the work of Voit [518] on the effect of iodothyrin on dogs. He was unable to maintain a positive nitrogen balance during the administration of iodothyrin even when large amounts of fat were used, and concluded that iodothyrin brought about its effect by its direct action on the nitrogen metabolism and not indirectly through loss of fat.

Later observations, however, suggested that other factors influenced in a quantitative manner the clinical status in hyperthyroidism in man. The increased protein breakdown was explained by the condition of undernutrition, particularly the lack of carbohydrate which generally characterized hyperthyroidism. This condition itself would bring about protein breakdown.

The first viewpoint concerning the influence of the thyroid on protein metabolism was therefore modified. The changes in the protein metabolism were believed to be the result of the amount and type of food consumed, and not the effect of the direct action of any toxic substance.

Schöndorff believed that following thyroid administration the increased requirements were at first met through use of body fat; this was shown by the decrease in body weight and increase in oxygen requirement. Only when carbohydrate reserves and fat were reduced was the protein attacked. Mayerle has pointed out that although the administration of thyroid material increased the nitrogen output and brought about a loss of weight, an increased intake of carbohydrate inhibited this effect and resulted in a sparing of the protein. An increased intake of fat spared protein in the same way. May had previously shown that even the loss of protein which is the usual concomitant of fever can be prevented by an adequate diet.

Rudinger demonstrated the fact that the minimal requirement of protein to replace the nitrogen of the wear-and-tear quota in hyperthyroidism was not above normal provided sufficient carbohydrate was added to the diet.

Grafe agreed with the statement made by Falta that in hyperthyroidism there is only an increase in the volume or intensity of the normal physiological reactions. It would therefore appear that there is no characteristic toxic effect of the thyroid on protein.

Steyrer was able to bring about nitrogen equilibrium in hyperthyroidism. The protein content of the diet was decreased somewhat, and at the same time sufficient calories were given to meet the require-

ments of the basal metabolic rate of the patient. When the protein was lowered still further, there was a negative nitrogen balance.

It is interesting to note that Müller, who first pointed out the increased nitrogen elimination associated with hyperthyroidism, has recently demonstrated in his clinic through the work of Lauter and Jenke that it is possible to maintain a nitrogen equilibrium in exophthalmic goiter where the metabolic rate is 80 per cent above normal provided sufficient carbohydrate is given.

Boothby and Sandiford [67] have confirmed earlier observations which showed that no one class of compounds is affected by thyroid activity: there is an increase in the carbohydrate and fat metabolism associated with hyperthyroidism. Furthermore they found that it was possible to maintain at least nitrogen equilibrium and usually to obtain a positive nitrogen balance provided sufficient carbohydrate and fat were administered, with a protein intake of between 1 and 2 grams per kilogram of body weight. In a few instances a little more than 3 grams of protein for each kilogram of body weight was required. The proportion of protein was, however, very low when compared with the mass of food taken. These results are evidence that the cause of the increased basal metabolism cannot be due primarily to an increase in protein breakdown and they suggest that the animal organism consumes at an accelerated rate whatever type of food is furnished. In none of the experiments was there any evidence that fat, carbohydrate or protein was burned in a qualitatively abnormal manner.

It may therefore be concluded from the work of many investigators that no product of the thyroid has a direct toxic effect on protein in the body and that patients with exophthalmic goiter can be maintained in nitrogen equilibrium on a properly balanced diet provided sufficient calories are furnished.

THE RELATION BETWEEN ALTERATIONS IN BASAL METABOLISM AND NITROGEN ELIMINATION

The early work which was carried out on this subject definitely established the fact that protein breakdown is intimately associated with the activity of the thyroid, but the observations were not placed on a quantitative basis until more complete knowledge of the physiological influence of the thyroid and of the chemical nature of its active agent was available.

The problem of the relation between an increased rate of metabolism and an increased nitrogen elimination has been of great interest

since the early work of Müller, Mendel, Ord and many others, who showed that coincidently with an increased basal metabolic rate there was a marked increase in nitrogen output. Schöndorff believed this was due to increased excretion of urea and other nitrogen-containing extractives. Von Bergmann found that in the obese the nitrogen excretion increased directly after thyroid feeding and sank when the production of heat increased rather abruptly. The heat production continued to increase while the nitrogen output decreased to less than normal.

Magnus-Levy [333] pointed out that the excess protein elimination occurred during the first few days of thyroid medication and that later the body appeared to become accustomed to thyroid feeding. Through a study of a carefully chosen group of patients, and through the use of pure, crystalline thyroxine, Boothby and coworkers have carried out an extensive investigation concerning the physiological action of thyroxine.

Boothby, Sandiford, Sandiford and Slosse, and Deuel, Sandiford, Sandiford and Boothby [124] found that the protein breakdown was not continuously in proportion to the level of the basal metabolic rate, and that variations in nitrogen elimination occurred only during the transitional period as the basal metabolic rate was increased or decreased. If the basal metabolic rate is increased by continuous administration of thyroid material, protein is broken down and the nitrogen output is increased for a short interval; however, a high basal metabolic level may be continued without the maintenance of increased protein elimination. After the basal metabolic rate has become stationary, although at a level much above normal, the nitrogen elimination returns to normal provided sufficient calories in the form of carbohydrate and fat are furnished. Conversely it has been shown by Deuel, Sandiford, Sandiford and Boothby [124] that if the basal metabolic rate is decreased by the withdrawal of thyroid medication then there is a transitory decrease in nitrogen elimination and after a short period nitrogen equilibrium is again established. In addition [124] it was shown that when a protein-free diet was given there was a marked drop in the basal metabolic rate and when a protein diet was resumed there was a still greater increase in the basal metabolic rate.

This work has furnished quantitative evidence that there is no relation between the quantity of "active" thyroxine functioning within the tissues and the rate of protein elimination. Nitrogen metabolism depends upon the caloric intake in relation to the caloric requirements and the type of food given. There is a definite relation between the output of

nitrogen and the transition in the basal metabolic rate which is brought about by either an increase or decrease in the concentration of thyroxine which is functioning in the body.

THE RELATION BETWEEN THYROID ACTIVITY AND DEPOSIT PROTEIN

The earlier conceptions of "circulating protein" and "tissue or organic protein" which were introduced by Voit,[517] assumed that the tissue or organized protein was stable living bioplasm, in contrast to the circulating protein dissolved in the body fluids. The question whether protein material was metabolized directly in the tissues or only after incorporation with the living protoplasm has now become only of historic interest. The more complete knowledge of protein metabolism which has developed with quantitative methods for the determination of amino acids and other known blood constituents explains the probable mechanism by which proteins are absorbed, transported and converted into heat and energy.

Whether there is present in the body a large amount of reserve or "deposit" protein has not been definitely established. There are large deposits of fat and carbohydrate in the body and Folin has concluded that a portion of the protein is present as "reserve protein." For this quantity Lusk [318] suggested the name "deposit protein." The experimental method which had been used to estimate the amount of deposit protein was to determine the nitrogen elimination and from this to calculate the amount of protein broken down during the time in which the protein constituents in the body were not replenished.

By an analysis of the muscle of salmon after long inanition and muscle activity, Greene showed that about 30 per cent of the muscle protein is lost. This result indicates the presence of a large amount of deposit protein in the salmon.

When it became apparent that the administration of pure, crystalline thyroxine produced an increase in the nitrogen elimination, Deuel, Sandiford, Sandiford and Boothby [125] utilized this physiological reaction in a new method to investigate the deposit protein in the body. A normal person was placed on a constant diet with either a high or a low nitrogen content. The deposit protein was then altered by the injection of thyroxine. In such an experiment they have shown that, after a preliminary period of thirty days, during which a non-protein diet was taken, with the loss of 149 grams of nitrogen, there was still the same characteristic effect of thyroxine on both the total and nitrogenous metabolism. This was interpreted as evidence that there was still avail-

able deposit protein which could be influenced by the administration of thyroxine. From the total nitrogen lost it was apparent that the deposit protein and its combined water amounted to at least 16 per cent of the body weight. This is a much larger value for the percentage of deposit protein than had previously been considered probable.

THE EDEMA OF MYXEDEMA

When thyroxine was given to a patient with myxedema it was shown (Boothby, Sandiford, Sandiford and Slosse) that there was a definite relation between the total loss of water and the total loss of nitrogen. In three cases the nitrogen was 1.9, 2.0 and 1.9 per cent of the water. The suggestion was therefore made that "the edema of myxedema is an albuminous colloid fluid with a nitrogen-water ratio higher than the average of 1.1 per cent which is that of human serum and identical with that of egg white which contains 2 per cent of nitrogen." The nitrogen content of myxedema fluid is definitely less than that of muscle which contains over 3 per cent of nitrogen.

These results support the hypothesis that in hypothyroidism there is a retention in the body of an excessive amount of "deposit" protein which is associated with water and is responsible for the edema. These investigators did not find in persons with normal renal function marked changes in the non-protein nitrogen of the blood or in its various partitions after administration of thyroxine. A slight increase in blood urea and non-protein nitrogen in some cases was apparently due to slight renal insufficiency; in other cases of myxedema the renal function was markedly impaired and high blood urea values were obtained after administration of thyroxine.

These new investigations of the relation between nitrogen elimination and thyroid activity confirm with quantitative values some of the earlier conceptions and definitely show that the thyroid does not primarily control the metabolism of the proteins. It appears to increase the rate of oxidation of all types of metabolites, the kind depending on their respective availability. The marked increase in nitrogen elimination following thyroid medication is but a transitory adjustment to a different metabolic level, and a continued increased nitrogen elimination in hyperthyroidism seems to be due to an insufficient food intake as it can be adequately controlled by a sufficiently high intake of calories mainly in the form of carbohydrate and fat.

Sandiford and Sandiford have recently shown that if carbohydrate is administered in large amount so that nitrogen metabolism is sup-

pressed as much as possible, there is even under these conditions a readjustment in the nitrogen equilibrium after the administration of thyroxine, and the usual and characteristic temporary increase in nitrogen elimination is obtained.

THE INFLUENCE OF THE THYROID ON CREATINE, CREATININE, PURINES AND SULFUR

The fluctuations in the total nitrogen excreted, in the experiments just described, Deuel, Sandiford, Sandiford and Boothby [124, 125] varied between 1.75 and 10 grams of nitrogen daily; however, the output of creatinine, uric acid, amino acid nitrogen, and undetermined nitrogen, as well as neutral and ethereal sulfur remained practically constant. The fluctuations in the total nitrogen were due to changes in the amount of urea alone.

Krause and Cramer showed that although thyroid administered to man caused an increase in urea output there was no change in the creatinine excretion.

Janney and Isaacson [255] have carried out an extensive investigation on the action of the thyroid gland in relation to nitrogen metabolism. They showed that after thyroidectomy there was a decrease in urine purines and there was an increased excretion of purines in experimental hyperthyroidism. Purine excretion of a cretin was low, but that of an exophthalmic goiter patient was high. They also showed that the creatinine excretion was independent of the administration of thyroid.

Several investigators have found that the creatine output varies with thyroid activity. Denis found creatine in five cases of hyperthyroidism. Palmer showed the creatine output to vary with the clinical condition in hyperthyroidism. Takahasi, Beumer and Iseke, and Carson have shown that administration of thyroid caused an increase in creatine excretion. Iseke has shown in hyperthyroidism an increase in the creatine output; in hypothyroidism creatine may not be excreted. He believed that the appearance of creatine in the urine, after the administration of thyroid, may be relied on as an evidence of thyroid activity in the material used.

EFFECT OF AMINES AND IODIDES ON THE NITROGEN OUTPUT

Although the administration of thyroxine or thyroid material is followed by significant changes in the nitrogen metabolism, other substances have been shown to produce a similar reaction. Abelin [10] found that the injection of phenyl or oxyphenylethylamine in a thyroidectomized dog for six days produced a negative nitrogen balance which

continued even after administration of the amines was stopped. Accompanying the increased nitrogen output there was diuresis and loss of body weight. The physiological effects of these amines produced a condition which closely simulated that following the administration of thyroid, and it is interesting to note that the time relationships involved in the activity of these amines approach those which are observed with thyroxine. A negative nitrogen balance persisted for eight days after administration of the material. This indicates a profound alteration in the equilibrium which establishes the basal and the protein metabolism.

The administration of relatively large amounts of iodides has been shown to increase the output of nitrogen. Grabfield, Gray, Flower and Knapp report a 27 per cent increase in the average daily nitrogen excretion following the administration of sodium iodide to dogs. This increase was not accompanied by any change in the sulfur excretion. These investigators consider that the increase is due to mobilization and excretion of deposit nitrogen. After removal of the thyroid gland an increase in nitrogen did not occur when iodides were given. They suggest that the thyroid gland is necessary for the increase of nitrogen output after administration of iodide, and that the thyroid may have as a function, apart from its effect on the basal metabolic rate, the mobilization of deposit nitrogen.

These experiments were carried out on dogs: 100 mg. of sodium iodide for each kilogram of body weight was given on three successive days. Similar experiments, which I performed several years ago on dogs, showed that sodium iodide, when given in smaller amounts, did not cause any change in the excretion of nitrogen. Hesse had previously carried out similar experiments and was not at all inclined to associate the thyroid with the increased nitrogen elimination. An objection to the conclusions of Grabfield, Gray, Flower and Knapp may be made concerning the experiments with thyroidectomized dogs. They show that in some of the normal dogs there was but little response to the injection of iodide and the percentage increase in nitrogen elimination is an average result obtained with several dogs, some of which were quite negative. Since this is true of normal animals, it is possible that a larger series of experiments on thyroidectomized animals would show an increase in nitrogen elimination after administration of large amounts of sodium iodide. It may also be essential to use an animal for this type of experiment which can be thyroidectomized without trauma to or removal of the parathyroid glands.

PHOSPHORUS AND CALCIUM METABOLISM

The excretion of phosphorus is not markedly influenced in hyperthyroidism. A slight increase has been shown in some cases. Gröbly suggested that phosphorus is involved in the activities of the thyroid gland through the nucleo protein in the gland. He suggested that the altered phosphorus metabolism in exophthalmic goiter results in decreased formation of nucleoprotein in the gland and therefore a decrease in the retention of the thyroid secretion. The inability of the thyroid gland to retain its active agent produces the condition of hyperthyroidism. Gröbly concluded that phosphates should be given to patients with exophthalmic goiter in order to prevent the discharge into the body of excessive amounts of thyroid secretion. This viewpoint has not been substantiated or proved incorrect by further work, but it is improbable that the course of exophthalmic goiter can be markedly altered by the administration of phosphates.

Heath, Bauer and Aub have recently reported some interesting results concerning calcium metabolism. They have shown that on a calcium-deficient diet the calcium balance in exophthalmic goiter is negative. In some cases the excretion may be as high as five times the normal amount. They found that phosphorus excretion was also increased but not to the same extent. After administration of Lugol's solution the basal metabolic rate decreased and the calcium excretion approached normal. They furthermore showed that administration of thyroxine caused an increase in the calcium output.

CHANGES IN THE VISCOSITY AND REFRACTIVE INDEX OF THE BLOOD

Simultaneously with the increased elimination of nitrogen, phosphorus and calcium produced by the administration of thyroid, blood volume and the physical properties of the body fluids are affected. Willius and Haines, and Thompson have shown a marked increase in the plasma volume in myxedema after administration of thyroid.

Deusch [126, 128] showed that in hyperthyroidism the concentration of serum proteins is decreased and that in myxedema it is increased. In hypothyroidism the normal concentration of the serum was restored by the administration of thyroid material. By the determination of the refractive index or the viscosity, changes in the blood could be shown in cases of exophthalmic goiter. Of the two methods Deusch considered the refractive index the more reliable, but he concluded [127] that the determination of this physical property of the blood could not replace the determination of the basal metabolic rate as a diagnostic test.

Frowein confirmed the work of Deusch and showed that after long administration of thyroid material to man there was a decrease in serum concentration when determined by the refractive index.

Hellwig and Neuschlosz followed the viscosity of the blood after operations for goiter. They found that the viscosity first increased and then returned to normal. As the activity of the thyroid gland appeared to control the viscosity of the blood and since the technic involved was simple and cheap these investigators suggested the determination of the serum viscosity as a method for the estimation of thyroid activity which was even superior to the determination of the basal metabolic rate. Von Frey and Stahnke concluded that the viscosity test for hyperfunctioning of the thyroid gland was not satisfactory for diagnostic purposes.

Hammett [191, 192] has shown that the removal of the thyroid gland from rats brought about a decrease in the water content of the serum and an increase in the refractive index. This indicated a partial desiccation of the serum and confirmed the view that the thyroid gland is concerned in the fluid exchange of the body. Hammett [193] has published a summary of previous work on water balance, in which he showed that thyroid and parathyroid deficiency produced a definite tendency to anhydremia in the central nervous system, in the blood and in the skeletal system. This he attributed to the lowered nutritional level brought about by these conditions rather than to a direct or specific effect of glandular activity on the distribution of water.

Although the viscosity and refractive index of the serum are dependent on thyroid activity it is doubtful whether the thyroid gland can directly influence the physical characteristics of the blood. Changes in metabolic activity may bring about alterations in the distribution of water between the tissues and body fluids, but the absolute value which is established after equilibrium has been attained would probably vary within limits too wide to be of accurate diagnostic value.

Fujimaki and Hildebrandt have shown that thyroxine caused water and sodium chloride to be mobilized in the tissues and pass into the blood. The total volume of the blood may be increased to from 130 to 140 per cent of the normal. Associated with the hydremia was a diuresis and a rapid excretion of sodium chloride. This was followed by a minimal loss of sodium chloride. (See Table 12.) These investigators considered the primary action of thyroxine in this case to be peripheral, since section of the spinal cord at the seventh cervical did not stop the hydremia and diuresis. The kidney apparently played a pas-

TABLE 12. *The Effect of Thyroxine on the Distribution of Water and Sodium Chloride Between the Tissues and the Blood (Fujimaki and Hildebrandt).*

Time	Body Weight Grams	Erythrocytes	Sodium Chloride in Blood Per Cent	Urine Cubic Centimeters	Specific Gravity	Sodium Chloride in Urine Grams	Sodium Chloride in Urine Per Cent
0	2123	5,450,000	0.57	..	....	....	...
50 min.	1 mg. thyroxine intravenously						
3 hours	2066	4,450,000	0.535	43	1.009	0.236	0.55
6 "	1980	3,800,000	0.54	75	1.009	0.540	0.72
9 "	1944	3,850,000	0.56	25.5	1.012	0.260	1.01
12 "	1918	4,200,000	0.56	21	1.015	0.185	0.88
25 "	1848	3,800,000	0.55	42	1.018	0.0042	0.01
28 "	1825	4,200,000	0.56	14	1.018	0.001	0.007
31 "	1796	4,400,000	0.58	20	1.016	0.052	0.26
34 "	1780	5,000,000	0.58	9	1.030	0.0342	0.38
37 "	1749	5,100,000	0.59	8	1.028	0.0256	0.32

Time	Blood Volume in Per Cent of Original Volume	Amount of Blood-Grams	Total Sodium Chloride in Blood Mg.	Sodium Chloride in Per Cent of the Normal	Water in the Blood Derived from the Tissues	Sodium Chloride in the Blood Derived from the Tissues
0	100	153	872	100	.....	.....
50 min.	1 mg. thyroxine intravenously					
3 hours	123	188	1006	115	+ 90	+370
6 "	143	219	1183	136	+117	+717
9 "	142	217	1215	139	+ 33	+292
12 "	129	197	1103	126	+ 6	+ 73
25 "	143	219	1205	138	+ 91	+106
28 "	129	197	1227	141	— 3	+ 23
31 "	124	190	1102	126	+ 19	— 73
34 "	109	167	969	111	— 9	— 99
37 "	107	164	968	111	+ 15	+ 24

sive rôle. The experiment lasted from twenty-seven to thirty-six hours; the first effect was seen in two hours after the injection, although the basal metabolic rate was increased on the fourth day. These authors considered that the result might be due to nerve reaction which involved permeability of the capillary endothelium. They concluded that thyroxine did not attack the kidney directly but that it increased renal activity because of the mobilization of salt in the blood.

Naito [362, 363] has shown that the thyroid gland indirectly affected the activity of the kidney as an excretory organ. In an animal in which renal insufficiency had been previously established, increased thyroid activity hastened death; a decrease in thyroid action delayed death. Naito believed that the effect of the thyroid was through an increase in the "rest nitrogen" in the blood and that the thyroid did not act directly on the kidney. Other experiments were carried out with rabbits, in

which resistance of the kidney to potassium chromate was shown to be altered by thyroid activity. Decreased activity or absence of the thyroid gland decreased the resistance of the kidney; increased thyroid activity brought about by medication increased the resistance; however, administration of thyroid material in toxic amounts produced a decrease in resistance. The time required for the excretion of water and phenolsulphonephthalein increased if the thyroid was removed. With a slightly injured kidney, administration of thyroid material affected the rate of excretion of phenolsulphonephthalein, but not that of water.

All of the effects of thyroid activity on hydration and elimination of water appear to involve two factors, the metabolic activity in the animal, and the specific stimulating or toxic effect of an excessive amount of thyroxine on each of the various organs of the body. It is difficult to make a sharp division between these two effects: the results strongly indicate that all cells in the body are influenced by the presence or absence of thyroxine.

SUMMARY

The administration of thyroid material will relieve all of the symptoms of myxedema and will reduce the size of goiters. The administration of thyroid causes a decrease in the body weight and an increase in the elimination of nitrogen. There may be a retention of nitrogen and marked increase in body weight if small doses of thyroid material are given to a young hypothyroid experimental animal or cretin. It was at first assumed that the increase in the elimination of nitrogen produced by large doses of thyroid material was due to a specific effect of the thyroid on protein metabolism. This view has now changed. The minimal nitrogen requirements in the condition of hyperthyroidism are not above normal, provided sufficient carbohydrate and fat are given in the diet. There appears to be only an increase in the rate of the normal physiological reactions and no direct and sustained effect of the thyroid on protein metabolism.

It has been shown that the rate of protein catabolism does not coincide with the rate of basal metabolism after the administration of thyroxine. There is a transitory increase in nitrogen elimination when the basal metabolism is increased and there is a decrease in nitrogen elimination when the basal metabolism is decreased. This indicates a reciprocal relationship between the basal metabolic rate and the amount of deposit protein in the body. The edema fluid in myxedema contains about 2 per cent of nitrogen. This is higher than the protein content

of serum of man. It is less than the protein content of muscle and is about that of egg white.

The increased excretion of nitrogen after administration of thyroid is due entirely to the increased excretion of urea. Creatinine, uric acid, amino acid nitrogen, undetermined nitrogen or neutral and ethereal sulfates do not increase. There is an increase in the amount of creatine excreted. An increase in protein metabolism is produced by phenyl and oxyphenylethylamine. Although the amount required is greater the effect is similar to that of thyroxine.

The injection of large amounts of iodides causes an increase in the excretion of nitrogen. The thyroid gland does not appear to be directly involved in this reaction.

Phosphorus metabolism is not markedly influenced in hyperthyroidism, but there may be a negative balance in calcium metabolism. Changes in blood volume and in the viscosity of the blood are found associated with hypothyroidism. Thyroxine produces its effect on the distribution of water by its action on the tissues. The kidney is only involved secondarily.

Chapter 8

The Influence of the Thyroid on the Nerves

Some of the most marked symptoms observed in hypothyroidism and hyperthyroidism involve the nervous system. Plummer [401] has described the nervous phenomena associated with exophthalmic goiter as follows: "The entire nervous system seems to be in a status of hyperirritability and fatigue. The patient is restless, easily irritated, and subject to emotional outbreaks. The picture varies with the duration as well as the degree of intoxication and with certain factors that for brevity may be grouped under the heading 'personality.' The intoxication is in many ways comparable to acute and chronic alcoholism. Tears and laughter come without the patient's knowing why." From the earliest investigations of the thyroid attempts have been made to relate the activity of the gland to the response of the nerves. These investigations may be divided into three groups: the first is concerned with the effect of iodine, iodides, iodothyrin, iodothyreoglobulin and iodized proteins; the second is concerned with the influence of the thyroid on the response of the animal organism to other physiologically active compounds such as epinephrine, pilocarpine, pituitrine, and so forth; the third group is concerned with the "acute" experiments or the influence of long-continued administration of thyroid material.

THE EFFECT OF IODINE COMPOUNDS ON THE NERVOUS SYSTEM

Soon after the isolation of iodothyrin von Cyon [117, 118] carried out a painstaking investigation concerning the nerve and blood supply to the thyroid and the effect of iodothyrin on the nerves, heart and circulation. He concluded that the blood flow through the gland was under control of the thyroid and cardiac nerves. After the injection of relatively enormous doses of iodothyrin (from 2 to 3 grams), stimulation of the depressor nerve decreased the blood pressure to a value half that of normal, but before the injection of iodothyrin, stimulation of the same nerve produced a drop of blood pressure which was equal to only from a fourth to a sixth of the normal pressure. After the vagus

nerve had been paralyzed with atropine its sensitiveness to stimulation could be restored with iodothyrin. von Cyon concluded that iodothyrin was one of the most important cardiac toxins arising in the body and that its purpose was to maintain the normal level of excitability for the regulatory cardiac nervous system and to relieve and combat both the internal and external injurious, toxic effects on this apparatus.

The results obtained by von Cyon appeared to agree with and explain some of the clinical conditions associated with exophthalmic goiter and since this early period in thyroid investigation the influence of thyroid preparations on the nerves, heart and circulation has frequently been used as a criterion for thyroid activity.

von Cyon and Oswald carried out further work on the effect of iodothyreoglobulin and iodothyrin on the circulation. They treated iodothyreoglobulin with sulfuric acid and extracted the iodothyrin from the insoluble residue with alcohol. The residue insoluble in alcohol contained a small amount of iodine, but it did not affect the heart and nerves as did iodothyrin.

The vagus nerve paralyzed with atropine was not relieved by iodothyreoglobulin, but it was relieved either by iodine in alkaline solution or by iodothyrin.

Further work of this nature was carried out by Isaac and v. den Velden with solutions of iodized proteins and peptones which were injected into cats. From 2 to 6 cc. of 0.5 per cent solution was used and although iodine-free albumin did not give any particular alteration there was a striking effect of iodized albumin in amounts of from 0.3 to 1 cc. This produced a 60 per cent drop in blood pressure and a decrease in pulse rate with recovery in a few minutes. The reaction could be prevented by atropine or by section of the vagus, but in this case there was still a vasomotor disturbance. The effect on the vasomotor system was not apparent before administration of atropine or section of the vagus. All iodized proteins reacted in the same manner. When these substances were tried on a rabbit the effect was not seen. Bromoprotein did not produce any effect on the circulation. These investigators concluded that either iodine in the protein or the altered protein molecule brought about the effect in cats, and they suggested that von Cyon's experiments do not have the significance which he gave them, as the increased activity of the nerves can be shown with iodine preparations other than iodothyrin.

von Fürth and Schwarz [166] criticized the work of von Cyon: they did not find any direct effect of iodothyrin on the circulation.

Oswald [386] found that the effect of stimulation of the vagus and depressor nerves was markedly modified after an injection of iodothyreoglobulin. The effect of iodothyreoglobulin appeared to be proportional to its iodine content: if the percentage of iodine was low there was but slight effect. Iodothyrin produced an effect similar to that of iodothyreoglobulin, but its influence was not at all in proportion to the iodine content. Iodothyreoglobulin prepared from goiters varied in its activity with the iodine content. Oswald concluded that iodothyreoglobulin possessed the property of increasing the nerve tone in an "exquisite manner" and believed that this action could be related to clinical syndromes of hypothyroidism and hyperthyroidism. If iodothyreoglobulin was treated with formaldehyde, its effect was not diminished. Oswald showed that other iodine-containing compounds found in nature did not affect the nerves as did iodothyreoglobulin. Iodized serum protein did not have any action; thyroid proteins other than thyreoglobulin and a protein-free extract of the thyroid had no effect. From these results Oswald concluded that investigations with iodothyrin did not disclose anything about the genuine thyroid secretion, and that iodothyreoglobulin is the active constituent of the thyroid gland.

The experiments of von Cyon [117, 118] and Oswald [385, 386] and others, concerning the influence of iodine compounds on the nervous system were performed before any quantitative values had been placed on the amounts of thyroid secretion necessary to produce a physiological effect. It has now been demonstrated by quantitative measurements that the total amount of thyroxine which is necessary to maintain a normal physiological status in the adult human being is less than 15 mg. Results which are obtained by the injection of two or more grams of iodothyrin, or correspondingly large amounts of other materials, cannot be regarded as of any value for the demonstration of the influence of thyroxine or of any iodine-containing compound in the thyroid, on the nervous system. Other objections which are inherently associated with this type of experiment will be pointed out in another section.

THE INTERRELATIONSHIP BETWEEN THYROID AND SUPRARENAL

In 1908, Kraus and Friedenthal investigated the effect of thyroid material on the physiological response to epinephrine. In addition, they carried out a series of clinical observations and have attempted to deduce the pathogenesis of exophthalmic goiter from the experimental and clinical work. The experiments of von Cyon were repeated. Iodothyreoglobulin was found to restore the excitability of the nerves to the heart

and vagus fiber paralyzed by atropine. From this work they concluded that the thyroid affected the tonus of the vagus nerve and this suggested the investigation of the effect of thyroid material on epinephrine, since it had also been shown to affect blood pressure. When juice from the thyroid gland was injected intravenously with epinephrine the action of epinephrine was found to be markedly prolonged. Kraus and Friedenthal concluded from these experiments and from clinical observations that in exophthalmic goiter there is a correlation between the thyroid gland and the suprarenal. They believed that although the suprarenal was secondary to the thyroid, its influence in the condition of exophthalmic goiter was important.

Asher and Flack apparently showed a correlation between the suprarenal and thyroid. They found that the thyroid gland secreted some substance which increased the irritability of the depressor nerve and increased the physiological action of epinephrine. They concluded that iodothyrin could not be the active constituent of the thyroid gland since it did not bring about the action on the nerves which was seen after stimulation of the gland. They also suggested that under physiological conditions the thyroid secretion, or extracts from the gland, had no effect on the pulse rate or blood pressure. This they believed was evidence that the symptoms of exophthalmic goiter were produced, at least in part, by the suprarenal gland.

Asher criticized the technic employed by von Cyon which was used to show the effect of iodothyrin on the nerves and circulation. He [27] later reviewed the question of the relation of the thyroid, the nerves and the suprarenals and considered the work of Eiger the most conclusive demonstration of the interrelationship between the thyroid and suprarenal.

Eiger [142, 148] used the Läwen and Trendelenburg [498] method to study changes in the perfusion rate through the leg of a frog. By this procedure he could show an interrelationship between some agent in the thyroid secretion and epinephrine. The addition of one part of epinephrine in five million parts of solution had no effect on the perfusion rate of the leg of a frog, but when this solution was combined with an extract of the thyroid gland the rate of perfusion was markedly decreased. On the other hand when the extract of thyroid was used alone there was no change in the normal perfusion rate. If instead of an extract of thyroid a solution containing the serum of a rat which had been fed thyroid material was used, then amounts of epinephrine too small to affect the normal rate of perfusion through the leg of the frog

caused a decreased flow. The normal plasma of rats, dogs and human beings, after three to five hours' oxygenation, exerted little if any effect on the perfusion rate of the vessels of a frog. The plasma from a patient with exophthalmic goiter did not influence the effect of a dilute solution of epinephrine, but it did enhance the effect of this substance if larger amounts of the latter were used. Perfusion of the leg of the frog with plasma from the inferior thyroid vein of a dog or from patients with goiter showed no direct effect, but the plasma increased the action of epinephrine and brought about marked constriction of the vessels.

Several years later Csillag carried out a further investigation with similar technic in which she found that thyroid extracts even in very great dilution enhanced the epinephrine effect in the perfusion of the frog's leg. Eiger's results were confirmed; however, it was pointed out that Eiger's method for the detection of thyroid substance was not reliable since the blood plasma of normal puppies, in and of itself ineffective, was found to enhance the epinephrine effect in the perfusion of the frog's leg. The blood plasma of puppies without thyroid glands, manifested this epinephrine-sensitizing property and human plasma not only of goitrous patients, but of normal individuals taken from the median vein was strongly effectual. The power of the plasma to sensitize epinephrine was not destroyed after it had been heated for one hour at 60°.

In 1916, Oswald [385, 386] showed that the injection of iodothyreoglobulin enhanced the effect not only of epinephrine but also of pilocarpine, pituitrine and other drugs, on the blood pressure and the pulse rate. In connection with a study of the interrelationships of the ductless glands, which was carried out by Cannon, Levy obtained an increased effect on blood pressure with epinephrine following stimulation of the nerve to the thyroid gland. Furthermore, the injection of a small amount of epinephrine, followed by a brief delay, caused a greater response to subsequent injections of epinephrine. This enhanced action was attributed to stimulation of the thyroid gland by the first injection of epinephrine. This effect was manifested only after a latent period of from forty to sixty minutes. Previous removal of the thyroid gland prevented this action. He believed that stimulation of the nerve to the thyroid caused it to secrete promptly, as thyroidectomy immediately after stimulation was followed by progressive augmentation similar to that observed when the thyroid gland was not removed. Levy showed that thyroxine brought about a similar effect but with this compound

the latent period was shorter. Stimulation of the thyroid did not change the blood pressure or pulse rate. The thyroid seemed to exert a selective action since the increase in vascular response was not associated with greater susceptibility of the augmentors of the heart.

Goetsch utilized the experimental results which indicated a relation between the thyroid gland and suprarenal glands and suggested the use of hypodermically or intradermically administered epinephrine as a clinical test for hyperthyroidism. He assumed that in hyperthyroidism there was a hypersensitiveness to epinephrine and concluded that a greater physiological response should be apparent in cases of excessive thyroid activity. The response of patients with goiter to the injection of epinephrine has been used as a clinical test of the severity of the condition, but Van Wagenen, Peabody, Sturgis, Tompkins and Wearn, Russell, Millet and Bowen,[437] Sandiford[441] and others have shown that the results of this test are questionable. While it is not the purpose in this monograph to review in detail the clinical aspects of goiter it may be stated in brief that this test has been shown to be so variable in different types of goiter and other clinical conditions that it is now regarded as of little use in the diagnosis of hyperthyroidism.

Recently further investigations of the relation between thyroid and suprarenal activity have explained some of the discrepancies which a review of the literature makes apparent. Collip has shown that the depressor effect of small doses of epinephrine in an animal under light ether anesthesia might be converted into pure, pressor effect by increasing the anesthetic. The same result was produced by increasing the pH of the blood with sodium carbonate. A pressor effect of epinephrine was increased by increasing the pH, and was decreased by lowering the pH.

Burget and Visscher found that in the pithed cat with constant artificial respiration small intravenous injections of epinephrine acted with progressively greater effectiveness, provided the alkalinity of the blood was increased. The physiological response to epinephrine was found to increase progressively as the pH of the blood was increased from the value 6.9 to 8.0. They furthermore found that the most effective way to vary the alkalinity of the blood was to change the amount of air carried to and from the lungs. They concluded that the secretion of the thyroid played no part in the increased activity of epinephrine, and that the increased responses to epinephrine were not due to the sensitization of the sympathetic nervous system by the thyroid. The increased response to epinephrine associated with an increased pH of

the blood appeared to be due either to an increased irritability of the sympathetic nervous system or to the more rapid oxidation of epinephrine brought about by the higher alkalinity of the blood. Burget and Crisler believed that variations in the response of the vascular system to epinephrine with changes in the pH of the blood were due to alterations in the irritability of the myoneural juncture rather than to any change of effectiveness brought about by variation of the susceptibility of epinephrine to oxidation.

Dryerre [136] could not obtain a consistent increase in the effect of epinephrine on the blood pressure after an injection of thyroxine. In fact, when he used a solution of sodium hydroxide of similar strength to that in which the thyroxine was dissolved he obtained an enhanced

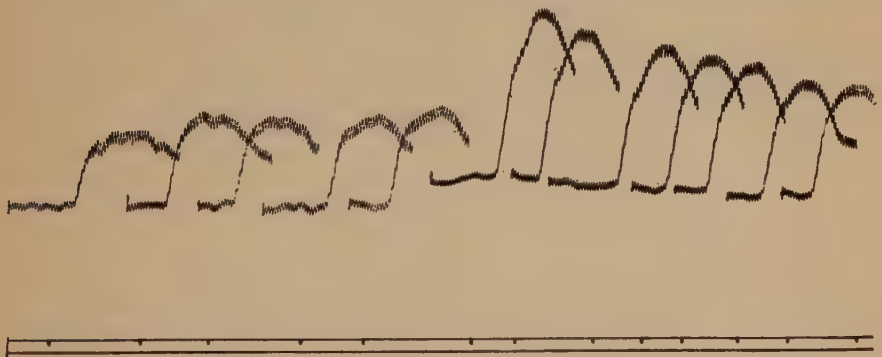


FIG. 25.—Blood pressure of a cat. The increases in blood pressure correspond each time to an injection of 0.003 mg. of epinephrine. Between the fifth and sixth injections 4 c.c. of a 0.1 sodium carbonate solution was injected. (Feldberg and Schilf.)

action to subsequent injections of epinephrine, which was similar in magnitude to that produced by the solution of thyroxine. Dryerre remarked that it was scarcely conceivable that the exceedingly small amount of alkali present in these injections could appreciably affect the hydrogen ion concentration of the blood.

Feldberg and Schilf found that the injection of 4 cc. of a 0.1 per cent solution of sodium carbonate greatly enhanced the effect of subsequent injections of epinephrine. (Fig. 25.)

The results of Dryerre and Feldberg and Schilf indicate that the presence of small amounts of either sodium hydroxide or carbonate may bring about a physiological response which is not at all in proportion to the change in the hydrogen ion concentration of the blood. This may be due to a temporary change in the acid-base equilibrium in cer-

tain portions of the body which are also sensitive to epinephrine. Injection of alkali, preceding the epinephrine, may increase the reactivity of this substance through a specific effect of the alkali on these centers which is greater than its effect either on the pH of the blood or on the other portions of the body.

Biological evidence for the presence of minute traces of the secretions of the thyroid and suprarenal glands is of undoubted value and with the types of experiments which have been described it is possible to show an interrelationship between substances in the thyroid and the activity of epinephrine and other compounds. Interpretation in terms of chemistry, however, is a difficult matter. Whether the increased action of epinephrine is due to thyroxine or any iodine compound in the thyroid, or is due to some other constituent of the gland which may occur widely distributed, has not been shown. The blood pressure changes are, evidently, due to many factors and the augmentation or inhibition of the effect of epinephrine may not be due to variations in the activity of the thyroid gland only.

"ACUTE" AND LONG-CONTINUED EXPERIMENTS

The condition present in the hyperthyroid individual must be regarded as chronic. The clinical history in many of these cases shows that the disease has frequently lasted for many weeks or months, and in some cases even years. It becomes apparent that all attempts to produce this condition by an "acute" experiment which is carried out in the course of a few minutes or hours are attended with uncertainty: it cannot be taken for granted that a temporary status of hyperthyroidism has been produced. "Acute" experiments have been carried out notably by Asher and Flack, who suggested that the substance which increased irritability of the depressor nerve and the effect of epinephrine on blood pressure is discharged from the gland after stimulation of the superior and inferior laryngeal nerves. Removal of the thyroid prevented an enhanced action of epinephrine even though these nerves were stimulated, but subsequent injection of thyroid substance produced the same effect as stimulation of the nerve to the gland. This result, they believe, proved that the thyroid gland has an inner secretion.

Still more recently, 1926, Herzfeld carried out experiments of both kinds, "acute" and long-continued. He found that continued administration of thyroid material in rabbits and guinea pigs caused an increase in the pulse rate. Removal of the thyroid did not affect the cardiograms in the experimental animals to the same extent as is found in human

myxedema. Animals which were fed thyroid material for some time showed greater sensitivity to stimulation with an electric current.

For a measure of the response of the nerves Herzfeld proposed the following method: the cervical sympathetic nerve was dissected out and stimulated for fifteen seconds with an induction coil. The degree of stimulation was determined by observation of the dilatation of the pupil. The coils of the induction apparatus were changed so as to produce various degrees of stimulation and the response to each of several positions of the coils was determined. He found in acute experiments that thyreoglandol, obtained by perfusion of the thyroid, produced a greater effect on the ocular sympathetic nerve than did an extract of desiccated thyroid. Intravenous injections of calcium in rabbits that had been fed thyroid material decreased the response of the vagus and increased the response of the sympathetic nerves. Large doses of calcium produced increased irritability of the vagus. In thyroidectomized animals there was no uniform action. Subcutaneous injection of epinephrine did not produce a uniform effect on the heart in thyroid-fed animals when compared with similar experiments on normal guinea pigs. Herzfeld concluded that the thyroid gland affects the equilibrium of the vegetative nerves; therefore, the thyroid influences vegetative regulation and thus the total reactivity of the body.

In contrast to the results of von Cyon, Kraus and Friedenthal, Oswald, Asher and Flack, Herzfeld and others, Dryerre [135] in the course of "acute" experiments showed that the degree of response of a nerve to the same stimulus was not affected by either thyroid extracts or thyroxine. He also called attention to the fact that it was not practicable to determine the sensitiveness of the nerves by the technic employed by Oswald. Dryerre left the nerve at rest during the whole experiment in an electrode of the fluid type which had been designed by Lucas. Extracts of liver and spleen, and even Ringer's solution caused variations in the response to the stimulus of the nerve as great as those brought about by addition of extracts from the thyroid gland. Injections of an extract of the thyroid and of thyroxine had no specific action on the sensitiveness of the cardiac nerve fibers or the vagus or the depressor nerves, which could be demonstrated in the course of an "acute" experiment. Furthermore, in "acute" experiments with animals under ether, urethane or paraldehyde anesthesia, no uniform or consistently increased pressor effect of epinephrine could be shown after the administration of thyroid. Finally, Feldberg and Schilf carried out experiments with cats and puppies in which they investigated the action of thyroxine, thyreo-

glandol, extracts from thyroid tablets and desiccated thyroid. Epinephrine was injected in doses of 0.003 to 0.005 mg. They summarize their work by stating that in acute experiments of this kind thyroxine did not sensitize, (a) the blood pressure action of epinephrine, (b) the blood pressure lowering effect of vagus stimulation, (c) the pupil dilatation produced by stimulation of the sympathetic.

The probable explanation of the variations found in acute experiments which are not due to changes brought about by the injection of alkaline solutions is given by Abderhalden and Gellhorn,[1] who demonstrated a sensitizing effect on a beating strip of frog's heart muscle of an extract of the thyroid. They found that the same effect is also shown by extracts of other organs, by histamine, tyramine and diiodotyrosine. Furthermore, these same extracts and amines also increased the sensitivity of the animal organism to epinephrine. They, therefore, made the suggestion that the secretions of the ductless glands circulating in the blood stream are of themselves ineffective until after they have been activated by some substances of a comparatively simple chemical nature. Burn and Dale have shown that the effect of histamine was greatly influenced by epinephrine.

The physiological response of certain of the amines, which is altogether out of proportion to the weight of the material injected, indicates that physiological processes are carried out in a system which is exceedingly sensitive not only to the amine group but also to the configuration of the molecule which carries this group; until a clearer conception has been gained of the chemical reactions which occur in nerve and muscle tissue, physiological results obtained by use of these active agents cannot be explained in terms of chemistry.

STIMULATION OF THE THYROID GLAND THROUGH ITS NERVE SUPPLY

That the thyroid gland influences the nervous system of the animal organism cannot be doubted. Another aspect of this problem concerning the relation between the thyroid and the nerves deals with the innervation of the thyroid gland.

The work of Asher and Flack, who suggested that stimulation of the inferior and superior laryngeal nerves caused the thyroid to discharge its secretion, has been described. On the other hand, Brun, under Biedl's direction, showed that, contrary to the findings of Asher and Flack, the effect of stimulating the depressor nerve with the same strength of current was not at all constant. Repeated stimulation in one

and the same experiment was frequently followed by marked differences in the fall of blood pressure.

Cannon and Cattell, through a study of the action current of the thyroid gland (a difference of potential between the thyroid gland and the surrounding tissue), showed that stimulation of the sympathetic cord, and not the vagus evokes an action current after a latent period of five to seven seconds. They concluded that the non-medullated nerves distributed to the thyroid cells belong to the sympathetic and not the cranial division of the autonomic system, and that their effects were not indirect through alteration of the blood supply: they were indeed true secretory nerves.

An attempt to demonstrate the stimulation of the thyroid through the nerves was made by Cannon, Binger and Fitz. The peripheral end of the cervical sympathetic nerve of a cat was anastomosed with the central end of the phrenic. Physiological function was demonstrated by dilatation of the pupil which was synchronous with the respiration. Stimulation of the thyroid was apparently accomplished: the behavior of the animal was changed and the basal metabolism markedly increased. This experiment was repeated, but in some respects has not been confirmed by the later work of Cannon and coworkers nor by other investigators. Mills, through the use of cocaine to sensitize and stimulate the nerves to the thyroid found that this drug did not change the histologic picture of the gland and he concluded that the sympathetic fibers to the thyroid do not exert any secretory function. Marine, Rogoff and Stewart,[341] failed to produce exophthalmos by anastomosis of the phrenic with the superior cervical ganglion. After anastomosis of these nerves the lobes of the thyroid on both sides of the animal were the same size and color and histologically no differences were seen.

Burget concluded that the central end of the phrenic nerve when sutured to the peripheral end of the cervical sympathetic either does not form a physiological anastomosis, or, if it does, the stimuli thus carried were not able to increase the output of the thyroid secretion sufficiently to produce the pathological changes associated with Graves' disease in man. Removal of a section of the cervical sympathetic cord did not produce changes in the thyroid gland in the cat. This indicated that secretory fibers, if such fibers were present, did not play a part in the normal mechanism of thyroid secretion. Troell, moreover, failed to produce exophthalmos through stimulation of the sympathetic.

Other attempts to show nerve control of the thyroid gland have been carried out by analysis of the iodine and water content and distribution

of iodine between the acini and cells of the gland before and after stimulation of the nerves. Rahe, Rogers, Fawcett and Beebe, reported a decrease in the iodine content of the thyroid gland following stimulation of the combined vagus and sympathetic nerve, and Watts reported changes in the iodine and water content of the gland following stimulation of the cervical sympathetic or the thyroid nerves. He concluded that the loss of iodine can all be accounted for by the vascular changes. More recently, van Dyke [511] has repeated this work and found that interpretations of the functional activity of the thyroid gland based on changes in iodine content are untenable. He could not find a significant difference in the iodine content or the distribution of iodine and of water in the thyroid after stimulation of the nerves.

Still another method by which to study the response of the thyroid gland to stimulation has been the quantitative determination by means of the precipitin test (Hektoen, Carlson and Schulhof [98, 207, 209]), of the amount of iodothyreoglobulin discharged from the gland into the blood or lymph. Stimulation of the nerves to the gland, the injection of epinephrine or pilocarpine and massage all yielded essentially negative results. Even this sensitive test has not conclusively shown that stimulation caused a marked outpouring of this protein which carries the active agent of the gland. It was shown (Hektoen, Carlson and Schulhof [208]) that the thyreoglobulin content of the blood of the thyroid vein was not uniform in different dogs and that it varies in the same dog from time to time. Hicks [223] was unable to show an increased output of thyreoglobulin after stimulation of the sympathetic nerve of the thyroid gland in a dog.

Despite many efforts to demonstrate an outpouring of the thyroid secretion after electrical stimulation of its apparent nerve supply, no method has quantitatively proved that iodine or thyreoglobulin leaves the gland in significant amounts. Indirect evidence that there is a constant exchange of iodine and thyroxine between the thyroid gland and the tissues, is indicated by the remarkably constant value for the iodine content of the blood (Kendall and Richardson, and Veil and Sturm). Other evidence which indicates that the thyroid gland is involved in the maintenance of the iodine content of the blood is furnished by the work of Hudson, who showed that after thyroidectomy the iodine content of the blood was increased. The fact that thyroxine produces such a marked influence upon the basal metabolic rate suggests that a rigorous control of the amount supplied to the tissues must be maintained. It is hoped that more work concerning the factors which influence the outpouring

of the thyroid secretion will eventually establish the mechanism by which secretion of the thyroid gland is regulated.

SUMMARY

One of the most striking physiological effects of the thyroid gland is made manifest through its influence on the nervous system. The experiments which have been carried out in attempts to explain this phenomenon concern the investigation of three factors: first, the effect of iodine and iodine compounds on the excitability of the nerves; second, the influence of the thyroid on the response of the animal organism to epinephrine, pilocarpine and other physiologically active substances; third, the time required for a maximal physiological response to a single injection of thyroid. The early experiments were carried out with relatively enormous doses of iodothyrin and iodine compounds. These results cannot be regarded as significant.

An apparent increase in the activity of epinephrine and other substances has been produced by the administration of thyroid material. Later work has indicated that some of the effects which were at first attributed to the thyroid may be due to changes in the hydrogen-ion concentration and the response of certain nerve centers to epinephrine. Experiments of short duration cannot be employed in the investigation of the physiological activity of thyroxine and desiccated thyroid. Whether the fresh thyroid juice contains a substance other than thyroxine which may be regarded as a specific product of the thyroid gland has not been conclusively shown. No convincing evidence has been secured which indicates a release of the thyroid secretion after stimulation of the nerve supply to the gland.

Chapter 9

The Influence of the Thyroid on the Toxicity of Acetonitril

In 1904 Hunt carried out an investigation of the physiological activity of acetonitril and among other reactions he tested the toxicity of this substance after administration of thyroid material to rats and mice. The thyroid material was administered to determine whether an increased rate of oxidation in the animal organism would cause a more rapid liberation of hydrocyanic acid and, therefore, a greater toxicity of the nitril. Hunt found that administration of thyroid material to rats increased the toxicity of acetonitril, but in both the white and gray mouse the administration of thyroid substance markedly decreased the toxicity of this substance.

The first paper concerning this work was published in 1905, when our knowledge of the thyroid was but meager. The work of Notkin, Wormser and Blum had placed before investigators in this field a theory concerning the detoxicating power of the thyroid. It was significant, therefore, when Hunt [238] showed that the toxic effect of at least one substance, acetonitril, was decreased by the administration of thyroid material in the mouse. In his original publication, however, the detoxicating action of the thyroid was not stressed.

Following the first work on the relationship between thyroid activity and acetonitril, many investigations were undertaken in the hope that this reaction in the mouse could be used as a criterion for the presence of thyroid material. The recent papers of Hunt [239, 240, 241, 242] review in great detail this phase of thyroid investigation. This method has been rigorously investigated in order to determine the basis for the chemical reactions involved, and to explain the protection afforded by thyroid material against acetonitril. Hunt has shown that this substance was oxidized to formic acid and the nitril radical which was liberated was then excreted as thiocyanate. The toxicity of acetonitril in the animal organism appeared to depend on two factors: the velocity of oxidation of the methyl group to formic acid; the velocity of detoxication of the

cyanide group as thiocyanate. Since these reactions occur in the liver and tissues, which are at the same time carrying on the normal processes of oxidation of various metabolites, variations in the toxicity are to be expected and Hunt and others have shown that merely a change in diet brought about sensitization to the drug which resulted in a fortyfold increase in toxicity. Furthermore, it would appear that lack of vitamines and a state of inanition are important factors. Some investigators have felt that these changes in the sensitivity of the animal to acetonitril make the method of no avail, but Hunt [239] has pointed out that when precautions were exercised concerning the mice used (they should be littermates, if possible) and when the diet was controlled and was uniform, the decrease in the toxicity of acetonitril after the injection of thyroid substance was as striking and characteristic as was the variation in basal metabolic rate after the administration of thyroid.

Hunt originally attributed the protection which thyroid material afforded against the toxic effect of acetonitril to the influence of the active constituent of the gland upon metabolic processes. Trendelenburg [497] showed that the blood of thyroidectomized cats could protect mice against acetonitril and, since this action could not be due to protection afforded by thyroid substance, he assumed that, in the absence of thyroid function, toxic substances accumulated in the blood and that these were the agents actually responsible for the test. He attributed the favorable effect of ingested thyroid material to its content of toxic substances which had been absorbed from the blood by the thyroid gland, and he suggested that iodothyrin contained impurities which were the active constituent so far as the protection of mice against the nitril was concerned.

This radically different viewpoint was shown to be incorrect by the use of pure, crystalline thyroxine: this compound could not be regarded as a vehicle to hold toxic substances which furnished the protective mechanism, and yet as Hunt [239] and Miura have shown, it alone will increase the resistance of mice to acetonitril. This furnishes conclusive evidence that the interpretation given by Hunt from the very beginning of his study of this reaction is correct, and that the protection afforded by thyroid material depends on changes in the velocity of physiological processes which are carried out in the tissues.

Gellhorn concluded that there were no grounds for a detoxicating theory of the thyroid gland which could be based on changes in the toxicity of potassium cyanide, acetonitril, and propionitril; the toxicity of these substances to mice was in the proportion of 1, 5, and 120,

respectively. The relative toxicity might be due to the velocity of the hydrolysis of the nitril. This occurred much more rapidly with propionitril than with acetonitril. Thyroidectomized mice were less sensitive to potassium cyanide and to propionitril, but more sensitive to acetonitril. This might be related to the relative non-sensitivity to the lack of oxygen after thyroidectomy which had been pointed out by the Asher school. Although thyroidectomy increased the toxicity of acetonitril in the mouse, and removal of the thyroid decreased the toxicity of potassium cyanide and propionitril, this result could not be due entirely to changes in the amount of thyroid material active in the body since castration produced the same effect. He further showed that extracts from other glands caused an increase in the resistance of mice to acetonitril.

In reply to this work Hunt [242] has pointed out that the increased resistance conferred by the extracts of other glands, testis, ovary, prostate and mammary is quantitatively much less than that furnished by the thyroid. When subjected to the physiological test it was not difficult to distinguish between them.

Wuth found the acetonitril reaction for the determination of thyroid activity to be reliable when properly carried out. He furthermore investigated the action of other substances and showed that mice were protected by tyramine and di-iodotyramine. He did not give a quantitative relationship between the necessary dose of these amines and that of thyroid material, but from the weights of material used it is evident that large amounts of these amines were required to bring about a protection equivalent to that which was conferred by a minute amount of thyroid material. He showed that β-imidozole-ethylamine was inactive. Koch found that tetra-iodohistidine anhydride and iodotryptophane did not afford protection in the mouse against the toxic action of acetonitril. Von Zwehl found that there was no protection given to the mouse by feeding di-iodo-indolepropionic acid. Di-iodotyrosine administered by mouth had no effect on the toxicity of acetonitril.

Von Zwehl further showed that large amounts of di-iodotyrosine protected female mice against acetonitril. The lethal dose was from two to three times that required for normal mice. No such effect was observed in male mice. With this compound, therefore, further specificity of action is apparent. This limitation in the action of di-iodotyrosine may raise doubt in the minds of some investigators as to the significance of the observations; on the other hand, the results emphasize in a correspondingly marked manner the delicate balance of the physiological

reactions in the tissues which are influenced by the presence of these compounds. Von Zwehl confirmed Hunt's findings that the protective action of thyroxine was very much greater than that of di-iodotyrosine. The protective action of thyroxine was the same on both male and female mice.

When Hunt investigated the effect of thyroid preparations on the toxicity of acetonitril it was with the specific object of increasing the rate of oxidation occurring in the animal; at that time there was no conclusive proof that the physiological activity of the thyroid was in proportion to its iodine content, but as further work was carried out with the acetonitril reaction [243] it became more and more evident that the protection afforded mice against acetonitril was in proportion to the iodine content of the thyroid preparation. Koch showed that the products of acid hydrolysis of the thyroid varied in their activity when tested with the acetonitril reaction and that iodothyreoglobulin was equal to the original desiccated thyroid when compared on the basis of the iodine content of each.

All doubt concerning the significance of the iodine content of thyroid material and the physiological activity of the iodine-containing compound was finally removed when Miura and Hunt [239] showed that pure, crystalline thyroxine will protect mice against the action of acetonitril.

We must furthermore conclude that the action of thyroxine upon the toxicity of acetonitril is not merely due to the iodine *per se*. Hunt [239] has shown that no other iodine compound approaches thyroxine in its ability to confer immunity to acetonitril on the mouse. Di-iodotyrosine, which is actually contained as a component part of the thyroxine molecule, has very slight action when compared with thyroxine in the acetonitril test.

Hunt [239] has also shown that it is possible to identify preparations of desiccated thyroid to which iodine compounds have been added. He was able to evaluate the activity of the thyroid material derived from goiters which showed various degrees of activity when examined histologically, and, although other substances have been shown to influence the reaction, it appears certain that the most practical and valuable test for thyroid activity is the protection afforded to mice against the toxic action of acetonitril. It is now certain that the measure of activity of thyroxine is the same whether tested by the acetonitril reaction, the basal metabolic rate or the relief of the hypothyroid condition.

Thyroid material has been shown to be present in the blood of patients with hyperthyroidism and in other diseases by means of Hunt's

test.[239] Salomon [440] has recently compared the blood in three clinical types of hyperthyroidism with normal blood and shown a positive reaction in the acetonitril reaction. Hunt [239] has pointed out that many related problems, such as the action of the vitamines and the significance of substances found in the blood and tissues in the body, might be studied by means of the effect of these substances on the acetonitril test. The method should give information of the greatest help in the interpretation of the biological significance of the chemical groupings. Since the nitril group appears to be detoxicated with formation of thiocyanate, sulfur metabolism must be involved and the detoxicating action of

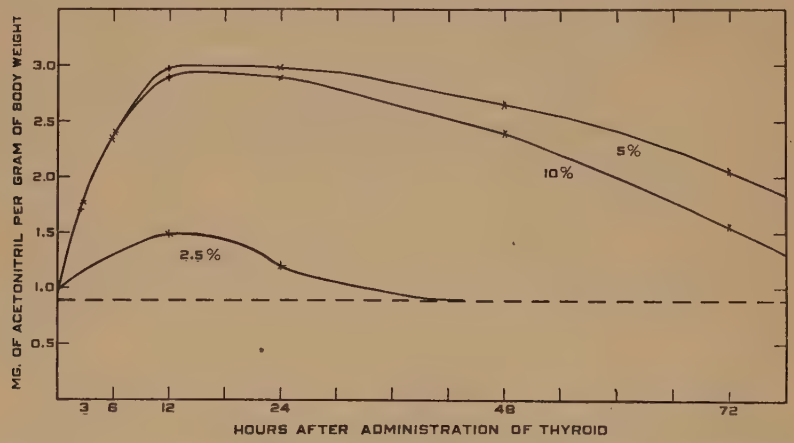


FIG. 26.—The curves show the resistance to acetonitril resulting from injections of 0.2 cc. of 2.5, 5 and 10 per cent suspensions respectively of desiccated thyroid. (Straub.)

glutathione and other sulfur compounds must be considered in connection with the test.

Straub [469] investigated the action of thyroid material on the acetonitril test and suggested that the procedure should be standardized in order to give more striking results when various samples were compared. As a unit of thyroid substance, he chose the smallest amount of dried thyroid which when given as one dose by mouth would produce a 100 per cent increase in tolerance to a subsequent intravenous injection of acetonitril. Two-tenths cc. of a 5 per cent suspension of thyroid material gave better protection than the same volume of a 10 per cent suspension; 0.2 cc. of 2.5 per cent suspension was ineffective. He showed that when the threshold dose was reached no more protection was afforded by larger amounts of thyroid material. (See Fig. 26.)

Hunt [241] has proposed a modification of this method to the extent that both the standard and unknown preparation should be tested on the basis of their iodine content and has suggested that the standard be desiccated thyroid of known origin containing 0.2 per cent of iodine. Furthermore, doses of unknown thyroid preparations should be based on their iodine content. It then follows that if the preparations are normal unadulterated thyroid, the relative strength, and so the correct dosage, is given at once by the weight of material administered in the test. If, however, the amount of iodine administered in the unknown sample afforded less protection than the standard it would indicate that part of the iodine in the unknown was not present in the form of combination specific for the thyroid.

SUMMARY

Thyroid material increases the resistance of the mouse to acetonitril. This reaction is confined to the mouse; no explanation has been found for this physiological relationship. The reaction is dependent upon the diet and other factors, but under uniform conditions it furnishes a method for the assay of thyroid material. No other gland affects the toxicity of acetonitril as much as does the thyroid. The activity of the thyroid is in proportion to its iodine content: thyroxine alone will protect the mouse against acetonitril. No other iodine compound brings about this reaction when given in the same concentration. Di-iodotyrosine will protect female mice to a slight degree; it has no effect on male mice.

Chapter 10

The Thyroid and Basal Metabolism

The clinical use of desiccated thyroid and the study of hyperthyroidism demonstrated in a very convincing way that the basal metabolic rate is markedly influenced by the thyroid gland. The investigation of Müller,[360] on the loss in body weight and the increased breakdown of proteins, was followed soon after by the work of Magnus-Levy [328] who showed that the consumption of oxygen and the output of carbon dioxide were increased in an obese individual after the ingestion of desiccated thyroid. No case of myxedema was available for treatment with thyroid material, but at this time Magnus-Levy also showed that in three cases of hyperthyroidism the oxygen consumption and the carbon dioxide output were much greater than in three normal individuals. These observations were made before Baumann discovered that iodine was normally present in the thyroid gland. Immediately after Baumann's discovery, Magnus-Levy [329] determined the effects on patients of desiccated thyroid, Fränkel's thyreo-antitoxin and iodothyrin. As criteria he used body weight, oxygen consumption and carbon dioxide output, and the pulse and respiration rates. Fränkel's [161, 162] thyreo-antitoxin did not affect the patient in any way, but desiccated thyroid and iodothyrin both increased the basal metabolism. The respiratory quotient decreased as the effect of the thyroid material became pronounced. Magnus-Levy [330] soon showed that in a patient with myxedema there was, after thyroid medication, an increase in oxygen consumption and carbon dioxide output and a change in the respiratory quotient. Among the several investigations carried out by this same investigator on the respiratory metabolism may be mentioned two others. In 1897 he [331] pointed out that the loss in body weight following the administration of thyroid material was not all due to loss of water and protein; there was also loss of fat.

The increase in the basal metabolic rate after the administration of thyroid was greater in a patient with myxedema than in a subject with a normal functioning thyroid gland. If the basal metabolic rate was raised

high above normal, a protein breakdown was made manifest by a negative nitrogen balance even with excessive intake of food. Iodothyrin was shown to bring about qualitatively the same effect, and finally it was suggested that the loss of protein and fat, which was brought about by the administration of thyroid material, was similar to the clinical condition observed in cases of exophthalmic goiter.

A few years later Magnus-Levy [332] gave the results of the administration of desiccated thyroid, iodothyrin, thyreo-antitoxin, and potassium iodide on cretins and myxedematous patients. These results were expressed in the percentage by which the basal metabolism varied from that of normal individuals. (See Fig. 27.) He showed that there were great changes in the oxygen consumption and in the carbon dioxide out-

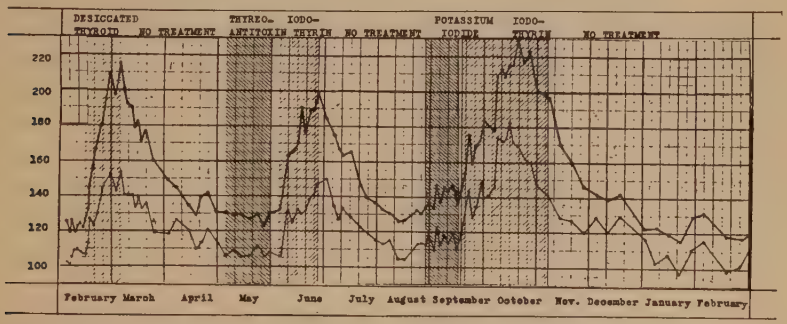


FIG. 27.—The influence of desiccated thyroid, thyreo-antitoxin and iodothyrin on the basal metabolic rate. Top curve oxygen consumed, bottom curve carbon dioxide produced in cc. per minute. (Magnus-Levy.)[332]

put. The basal metabolism rose to 55 per cent above normal. In the bibliography of this paper there were but two articles concerned with respiratory metabolism besides his own work. This emphasizes the fact that practically all of the pioneer work in this most important phase of thyroid investigation was carried out by Magnus-Levy.

Work on respiratory metabolism was continued by Hirschlaff, von Bergmann, Salomon,[439] and Steyrer. They showed that there was an increase in the basal metabolic rate either in the condition of hyperthyroidism or after the administration of thyroid material. The early investigations of the respiratory metabolism have been gathered together by Du Bois, and will not be given in detail here. Respiratory metabolism has been reviewed by Magnus-Levy, [333] and by Grafe, and more recently by Boothby and Sandiford.[68]

In 1915, Means investigated the basal metabolic rate of a group of

patients with pituitary and thyroid disturbance. In 1923, Boothby and Sandiford [67] investigated the total metabolism in cases of exophthalmic goiter and found that the caloric intake required to maintain a positive nitrogen balance and prevent loss of weight was as high as 5,000 calories. This is in excess of the amount given to soldiers on active duty in the field: it indicated that in some manner the energy expended in performing work was greater than that required in the normal individual. This hypothesis, which was suggested by Plummer, was proved to be correct by the determination of the cost of work when performed by a patient with hyperthyroidism. The number of calories expended in walking a given distance was about twice that required by the normal individual. When this value was used in the calculation of the calories required, the number of calories contained in the food and the number of calories expended by the known activity of the patient very nearly balanced.

Sturgis at about the same time made many observations at frequent intervals in one case of exophthalmic goiter. The limit of error in these determinations was between 3 and 5 per cent. He showed that the body weight may remain stationary, increase or decrease, irrespective of the level of the basal metabolic rate, and that the caloric value of the diet necessary to maintain equilibrium might be as high as 85 per cent above normal.

Du Bois concluded that the measurement of heat production gave the best index of the severity of the hyperthyroidism and that it also provided the best means of following the effect of treatment. In a series of eleven cases he found that the basal metabolism was from 50 to 75 per cent above normal. Russell, Millet and Bowen concluded that the basal metabolic rate afforded the best index of the degree of thyroid activity. Boothby [62] has suggested the fundamental classification of disease by the basal metabolic rate and [61] has shown the influence of various forms of hyperthyroidism on the basal metabolic rate. Boothby and Sandiford [68] have shown, in several thousand cases of hyperthyroidism, the value of this determination. Jordan found that the clinical results of surgical treatment are consistently indicated by the changes in the metabolic rate. Between 1915 and 1925, following the work of Benedict, Means, Du Bois, Boothby and Sandiford, and others, there was a remarkable increase in the use of the basal metabolic rate determination in clinical medicine; this test is now available for the internist in all medical centers in this country.

THE INFLUENCE OF THYROXINE ON THE BASAL METABOLISM OF EXPERIMENTAL ANIMALS

In addition to the work on the respiratory metabolism which was carried out on human beings, the influence of thyroid material on animals has been investigated. Voit [518] showed that thyroid material administered to a dog produced from 16 to 20 per cent increase in the output of carbon dioxide.

Arnoldi has investigated the influence of thyroxine on the basal metabolism of the rat; after the injection of this substance there was a decrease in the basal metabolic rate during the first four hours. After seventeen to nineteen hours there was a pronounced increase. The immediate response of the animal depended on individual factors; injection of large amounts brought about a condition similar to hyperthyroidism with an increase in basal metabolism.

Sierens and Noyons investigated the effect of thyroxine on the basal metabolic rate of pigeons. They found that the dose administered influenced not only the intensity of the response but also the time of its action. The larger the dose the more rapid was the manifestation of its effect. Kunde and Kunde and Steinhaus have carried out an extended investigation on the influence of thyroid material and thyroxine on the basal metabolism of dogs.

THE INFLUENCE OF AMINES AND OTHER SUBSTANCES ON BASAL METABOLIC RATE

Knipping and Wheeler-Hill have investigated the influence of thyroxine and thyroid material on the basal metabolic rate, especially in regard to the fat-reducing power of these materials. They found that other amines and curare brought about specific effects in the animal organism but that thyroxine and epinephrine were the most active.

Abelin [10] has shown that phenyl and *para*-oxyphenylethylamine increased the carbon dioxide output and oxygen consumption. Small amounts of both of these amines for five consecutive days increased the metabolism from 16 to 20 per cent. These amines also caused increased loss of water and diuresis. Tyramine and phenylethylamine caused an increase in carbohydrate metabolism, during which the respiratory quotient approached 1. Increase in the gaseous exchange persisted after the administration of the material was stopped. A similar effect in regard to the basal metabolic rate was observed after the administration of thyroid material was stopped. *Iso*amylamine, when given alone, had no effect on the gaseous metabolism.

Abderhalden and Wertheimer [5] have recently investigated the influence of diet on the activity of thyroxine. They showed that if thyroxine was administered to rats while they were given a carbohydrate-rich, protein-poor diet, there was a relatively slight increase in respiratory metabolism which amounted to only 14 per cent, and the effect lasted but three days. When they were on a high-protein diet, administration of thyroxine brought about a marked increase in the gaseous exchange which amounted to 37 per cent and the increased respiratory metabolism lasted for over nine days. When they were on a high-fat diet thyroxine caused an intermediate effect which more nearly resembled that obtained with a high-protein diet; the highest increase in metabolism was 24.5 per cent and it lasted for six days. These results show that the products of protein metabolism are of the greatest significance to the activity of the thyroid gland. Abderhalden and Wertheimer questioned whether the products of carbohydrate metabolism could counteract the severity of the symptoms of hyperthyroidism. They have shown that a high-carbohydrate diet in cases of hyperthyroidism in man is helpful, whereas a high-protein diet aggravates the symptoms.

In addition to the influence of fats, carbohydrates and proteins, on the physiological response of thyroxine, Abderhalden and Wertheimer [5] found that ergotamine produced a striking effect on the physiological response of thyroxine. The clinical and pharmacological influence of this substance had been shown by Aldersberg and Porges, and by Rothlin. Abderhalden and Wertheimer showed that injection of ergotamine prevented thyroxine from bringing about its typical action over a period of days. Ergotamine caused a drop in both body temperature and body weight; continued administration was followed by less and less reaction, until eventually the body temperature was increased by an injection of ergotamine. If the administration of this compound was stopped for one or two days it was found that further injections brought about a result similar to the first effect.

Marine, Deutch and Cipra investigated the effect of ergotamine on the heat production of normal and thyroidectomized rabbits. They found that this substance appeared to react in an opposite way to epinephrine. It lowered the body temperature and the heat production in cases of hyperthyroidism; in the rabbit there was a striking fall in the heat production, with no untoward effects, following administration of ergotamine. The decrease in the basal metabolic rate was proportional to the dose. The basal metabolic rate in thyroidectomized animals was lowered but to a less degree than in the normal animal.

The work of the last two groups of investigators emphasizes the fact that the activity of the thyroid gland is dependent on specific chemical groupings. The degree of physiological response to the administration of thyroxine is determined by the condition of the animal organism, and the oxidative processes which are influenced are also affected by a quantitative relationship between the various metabolites, other amines, the degree of hydration of the tissues and many other factors. It is significant that no effect can be demonstrated immediately after the administration of desiccated thyroid or of thyroxine to an animal. There is a latent period before its effect on the metabolism becomes manifest. During this interval there is an alteration in the distribution of water (Fujimaki and Hildebrandt) and when the basal metabolic rate is increased there is a coincident change in the output of nitrogen. The significance of these variations in various equilibriums is not at present understood, but the facts which have been established by experimental observations are conclusive evidence that the effect of thyroxine on the basal metabolism is intimately bound up with chemical processes which involve oxidation.

QUANTITATIVE RELATION BETWEEN THYROXINE AND THE BASAL METABOLIC RATE

Although the results of Magnus-Levy [332] showed that the basal metabolism was greatly influenced by the administration of thyroid, other investigators obtained varying degrees of response to the administration of thyroid material, and a quantitative relationship could not be determined and, in particular, accurate information could not be gained concerning the amount of the active agent of the thyroid which was functioning in the body.

After the isolation of thyroxine in pure, crystalline form, the opportunity was afforded to determine the quantitative relationship between this substance and the basal metabolic rate and from such data to calculate the total amount of thyroxine functioning in the animal organism. Such an investigation was undertaken by Plummer in 1917, and later by Boothby. This work was carried out on normal human beings and on a large series of patients with myxedema.

I [271] had shown that after the injection of thyroxine in normal animals and man there was a latent period before its activity became evident; the same delay in its action was found throughout this series of cases of myxedema. Six or eight hours after a single injection of thyroxine there was a slight increase in the basal metabolism. This rather

rapidly increased but the maximal response to a single injection was not reached before the eighth or tenth day. After the maximal response there was a slow decrease in basal metabolism which did not return to normal until after three or, in some cases, four weeks.

It has been clearly shown that the injection of thyroxine causes a marked shift in several of the body constituents, and the slow return to the former basal metabolic rate may be due to the time required to re-establish the equilibrium which existed previous to the injection of thyroxine. Since the maximal response does not occur before the eighth to the tenth day it must be assumed that some inhibiting action delays the manifestation of the full physiological activity of the material injected. The physiological processes involved appear to possess the property of inertia and are resistant to a change in either direction.

Magnus-Levy [330] was the first to point out that in hypothyroidism the basal metabolic rate was approximately 40 per cent below that of the normal individual and in no case in the large series investigated by Plummer and Boothby have metabolic rates been found that were lower than about 45 per cent below normal (Du Bois standards). From the results of Plummer and Boothby it soon became evident that the injection of thyroxine in a normal human being was not followed by the same increase in basal metabolism as resulted from the injection of the same amount of material in a patient with little, if any, active thyroid gland. This observation confirmed the earlier work of Magnus-Levy [331] on the activity of iodothyrin in relation to a normal or hypofunctioning thyroid gland.

Through experiments on myxedema patients the variations which are encountered after the injection of thyroxine in normal human beings are eliminated, and, from the quantitative nature of the physiological response to thyroxine which has been shown by Boothby and coworkers, it is evident that the human being offers unique advantages for the study of changes in the basal metabolic rate. By the use of small injections of thyroxine, which appeared to be completely incorporated in the functioning protoplasm, Boothby, Sandiford, Sandiford and Slosse have shown that there is a surprisingly exact relationship between the amount of thyroxine injected and the subsequent basal metabolic rate. This was shown by three different methods: First, known amounts of thyroxine were injected in a large series of cases of myxedema. The average increase in basal metabolic rate per milligram of thyroxine was determined in each case. Secondly, known small amounts were injected either each day or every other day in a case of high-grade myxedema

and the elevation of the basal metabolic rate found. This furnished data for the determination of the amount of thyroxine destroyed daily. (See Fig. 28). Finally, thyroxine was administered intravenously to a normal individual on a restricted and carefully controlled diet, in order to determine the relationship between the nitrogen output and the basal metabolic rate. These results have been reported in a previous chapter. As a result of these investigations we may say that but few compounds are known the physiological response to which can be expressed in terms as exactly as can the response to thyroxine. The specific dynamic action of various

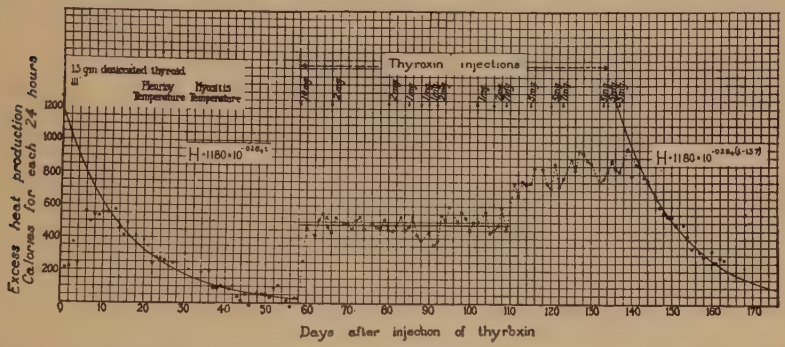


FIG. 28.—The influence of desiccated thyroid and thyroxine on the basal metabolism. During the first day, at the time indicated by the three arrows, desiccated thyroid containing 13 mg. of iodine was administered. If the iodine was all present in the form of thyroxine, the weight of the thyroxine was approximately 20 mg. On the 60th day 10 mg. of thyroxine was given, and between the 66th and 110th day the basal metabolism was maintained at a practically constant level by the injection of small amounts of thyroxine. Between the 110th and 140th days the basal metabolism was raised to a higher level by the injection of larger amounts of thyroxine. These results indicate that 10 mg. of thyroxine has practically the same effect on the basal metabolism as 3.9 g. of desiccated thyroid, which contains iodine equal to that in 20 mg. of thyroxine. (Boothby and Sandiford.)[69]

amino acids and of glucose has been shown to lie within narrow limits. The increase in blood pressure, after the injection of epinephrine or the active constituent of the pituitary, has been shown to depend quite consistently upon the amount injected but these results are of short duration. The elevation of the basal metabolic rate over a period of from two to three weeks is not brought about by any substance other than thyroxine.

Comparison of the results obtained by the injection of thyroxine in a large number of cases of myxedema showed that on the average 1 mg. of thyroxine produced an increase in the basal metabolic rate equivalent

to 2.8 per cent of its normal value. If large amounts were injected the increase for each milligram was somewhat less; this indicated that some of the thyroxine was eliminated before it was incorporated into the protoplasm in a functioning form. (Table 13.)

TABLE 13. *Increase in the Basal Metabolic Rate from Intravenous Injection of Thyroxine in 69 Cases of Myxedema (Boothby and Sandiford).*[69]

	Increase per mg. 2.8 Average variation, 0.7 = 25% Largest plus variation, 2.8 = 100% Largest minus variation, 1.8 = 64%				Increase per mg. per sq.m. 4.7 Average variation, 1.1 = 23% Largest plus variation, 2.9 = 60% Largest minus variation, 2.9 = 60%			
AVERAGE BASAL METABOLIC RATE	VARIATION IN CALORIGENIC ACTION DEPENDING ON THE ORIGINAL LEVEL OF THE BASAL METABOLIC RATE				VARIATION IN CALORIGENIC ACTION DEPENDING ON SIZE OF DOSE			
	Average dose	Increase per milligram	Average dose per square meter	Increase per milligram per square meter	Average dose	Increase per milligram	Average dose per square meter	Increase per milligram per square meter
Above to –14	8.7	3.3	5.7	5.0	4.8	3.5	3.6	5.0
–15 to –19	9.1	2.3	5.1	3.9	6.9	2.8	5.2	5.0
–20 to –24	7.3	2.9	4.6	4.6	8.8	2.9	7.0	5.2
–25 to –29	10.9	2.8	6.2	5.0	11.2	3.3	9.3	4.8
–30 to –34	14.8	2.9	9.2	4.9	16.0	2.6	11.5	2.7
–35 and below	15.5	3.0*	8.6	5.1	24.0	1.6	13.2	3.0

Loss of activity of thyroxine was shown after a single injection of large amounts of thyroxine in animals. One hundred and fifty mg. of thyroxine injected in a goat produced a marked effect, but after an interval of two or three days the animal returned to normal. If this same amount of material was divided into fifteen 10 mg. amounts, one of which was injected each day, it was frequently found that the goat died from a condition of hyperthyroidism before the last injection (Kendall [268]).

In any given case of myxedema, the rate at which the basal metabolism returns to normal after an injection of thyroxine, is not the same on each succeeding day. Boothby found that the rate when charted on coördinate paper was not a straight line. When the results were plotted on semilogarithmic paper they fell on approximately a straight line. Following up this observation, Boothby and Baldes [64] were able to derive an equation for what they have termed the decay portion of the curve. They have shown that the velocity followed an exponential curve which accurately expressed the rate at which the former metabolic rate is reached after an injection of thyroxine. The basal metabolic rate of

any patient treated with thyroxine can be predicted by this equation, provided the slope of the curve is established for each case.

In 1904 Magnus-Levy [332] reported observations on the change in the basal metabolic rate of a cretin after the administration of desiccated thyroid, and iodothyrin. Boothby and Baldes [69] calculated the slope of the decay curve in this patient and by means of the equation plotted the decay curve which would have been given if the basal metabolism had been increased by the administration of thyroxine. The decay curve for iodothyrin which had been determined and plotted twenty years previously by Magnus-Levy coincided with the predicted values to a surprising degree. (Fig. 29.)

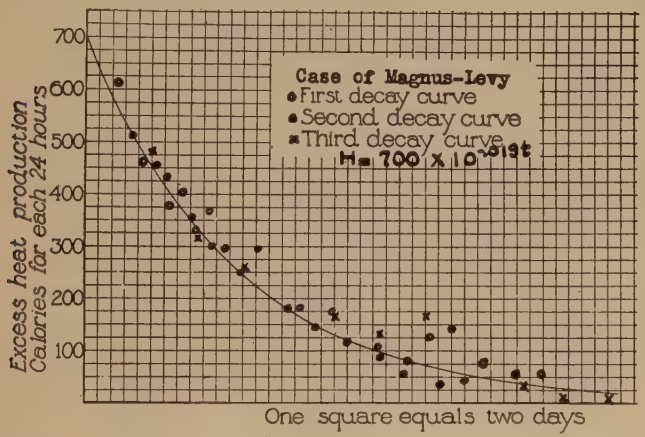


FIG. 29.—Velocity with which the basal metabolic rate returns to its former level after injection of thyroid material. (Boothby and Sandiford.)[69]

After it had been shown that the decay portion of the response to thyroxine was of an exponential order, an equation was derived to express the velocity at which the basal metabolic rate was increased after a single injection and an exponential equation was derived for this portion of the curve. With both the acceleration and decay portions of the curve expressed mathematically they then succeeded in combining these two equations into a single equation of two terms which can be used to predict the basal metabolic rate at any time following a single injection of thyroxine.[65]

Whether or not the decay curve is an actual measurement of the rate at which thyroxine is destroyed may be open to question, but the fact remains that the velocity of the processes which are altered by the

injection of thyroxine can be expressed mathematically. From the amount of thyroxine which must be injected each day in order to maintain a normal basal metabolic rate in cases of high-grade myxedema, Boothby and Baldes have calculated that the daily destruction is less than 0.4 mg. and that the total amount of thyroxine functioning in the body is between 8 and 15 mg., which agrees with Plummer's earlier estimation of 14 mg. Since approximately 40 per cent of the total energy produced in the animal organism when at rest is due to the presence of this amount of thyroxine, it is difficult to escape the conclusion that thyroxine functions as a catalyst. The other substances which are known to increase basal metabolism, epinephrine, amino acids, amines and glucose, all have but a transitory effect on the velocity of the oxidative processes in the body; on the other hand, thyroxine appears to be able to elevate and to maintain the rate of the production of energy on a much higher level for many days.

Kunde has recently criticised the work of Boothby and coworkers concerning the quantitative response to thyroxine. Her work was done on rabbits and dogs and the results make the fact still clearer that for this type of work the human being is far more desirable than experimental animals. The results of Boothby, Baldes and Sandiford on completely myxedematous (thyroidless) subjects advance our knowledge of the quantitative relationships in a definite, concrete manner, and their interpretation is not altered because similar results are not produced on animals such as the rabbit or dog, that possess intact and functioning thyroids.

The quantitative demonstration that thyroxine possesses unique power to control oxidative processes in the body, emphasized the fact that the determination of the basal metabolic rate in the human being in the absence of other disease may provide a method for the determination of the functional activity of the thyroid gland. An analysis of many thousand determinations of the basal metabolic rate in a large number of cases has shown that, other factors being standard, the basal metabolic rate is a reliable index of the activity of the thyroid (Plummer [401]).

Although this statement may not go unchallenged, the facts that have been established concerning the quantitative effect of thyroxine on the basal metabolic rate cannot be questioned, and justify the conclusion that the basal metabolic rate does measure the activity of the thyroid.

SUMMARY

The administration of thyroid material increases the basal metabolism. This test affords the best index of the activity of the thyroid gland. The determination of basal metabolism in clinical medicine is now carried out on an extensive scale. Certain amines, notably phenyl and *para*-oxyphenylethylamine, will increase the basal metabolism; *iso*amylamine is without effect. Thyroxine alone will increase the basal metabolism but the quantitative response depends on whether the diet consists largely of carbohydrates, fat or protein. The greatest response and the longest effect are produced when the diet consists largely of protein. Ergotamine will prevent thyroxine from bringing about its typical effect on the basal metabolism.

With a well-balanced diet thyroxine quantitatively influences the basal metabolism of man. In a patient with high-grade myxedema, 1 mg. produces an increase of 2.8 per cent of the normal value: from six to eight days are required before a single dose of thyroxine produces its maximal effect and the basal metabolism does not return to normal until after four or five weeks. An exponential equation with two terms has been derived for the curve which expresses the increase and decrease of the basal metabolic rate after an injection of thyroxine.

Chapter 11

The Influence of Lack of Oxygen on the Animal Organism

In 1917 Rippstein published an article from Asher's laboratory concerning the possible relationship between mountain sickness and lack of oxygen. The work was carried out on rats, which were placed in a glass compartment from which the air was removed, and the partial oxygen pressure was determined at which the rats showed a physiological response to the lack of oxygen. He showed that when air was in the compartment the effect began as soon as the partial pressure of the oxygen was 27.68 mm. of mercury; if the compartment was first filled with oxygen the partial pressure could be reduced to 23.48 mm. of mercury before an effect was noticeable. In an atmosphere largely of nitrogen the symptoms became manifest when the pressure was 29.10 mm. of mercury. Rippstein concluded that a decrease in the partial pressure of oxygen seemed to be an important factor in mountain sickness.

A short time after the work of Rippstein, Streuli further investigated the effect of lack of oxygen in relation to the activity of the thyroid gland. He showed that thyroidectomized rats were much less sensitive than normal rats to lack of oxygen. From experiments on the thyroid and the spleen, Streuli concluded that both of these organs were involved in the physiological response brought about by lack of oxygen.

Duran carried the work still further: rats which were fed thyroid material were more sensitive than the normal rat to lack of oxygen. He confirmed the observation of Streuli that thyroidectomized rats were less sensitive than normal. Duran suggested that the physiological response to lack of oxygen could be used as a method for the determination of the presence of thyroid material. This has been called the Asher test, and was applied as an assay of thyroid activity by Hara, who used white rats of uniform weight and injected 10 cc. of blood;—3 cc., 3 cc. and 4 cc. on the first, second and third days, respectively. The test as applied by Hara was carried out on five rats, one a normal, the second a rat given

10 cc. of normal blood, the third a rat given 10 cc. of blood from a patient with goiter, the fourth a rat given 10 cc. of blood from the thyroid vein, and the fifth a rat fed known amounts of thyroid substance for three days. The five rats after this preparation were placed in a jar from which the air was partially exhausted. From the results of experiments of this kind it was concluded that the method showed the presence of the thyroid secretion qualitatively, and to a certain extent quantitatively. The injection of normal serum had no effect. Feeding exophthalmic goiter glands produced a greater effect than the ordinary colloid goiter, and the parenchymatous goiter gland was more active than a cretin thyroid gland. However, the cretin thyroid appeared to have some activity. Blood from the thyroid vein was active and varied with the type of goiter; it was inactive when taken from a cretin's thyroid. Blood taken from the arm vein was weakly positive in cases of colloid goiter, but was inactive in cases of exophthalmic goiter. Hara concluded that cretin blood not only did not react as normal, but that it decreased the sensitivity to oxygen lack: he assumed that in the cretin blood there was some substance which was antagonistic to the thyroid secretion.

de Quervain investigated the clinical application of the Asher test based on the effect of lack of oxygen on the rat. A series of determinations were made with human blood from the arm and from the vein of the thyroid gland. de Quervain also found that the blood from the cretin caused a decrease in the sensitivity in rats to lack of oxygen, and assumed that in the absence of thyroid activity, products accumulated in the blood which neutralized the effect of the thyroid gland of the rat. The serum from a patient with exophthalmic goiter mixed with the serum from a cretin was less active than the serum from the same patient mixed with normal serum. While the results appeared to be definite, it was felt that they were too few to settle the question whether the blood of an individual deficient in thyroid activity actually decreased the sensitivity of a rat to lack of oxygen.

Pedotti and Branovacky found that there was not an exact parallelism between the basal metabolic rate of a patient and the results of the Asher test. The blood from the arm vein was given to rats which were then observed while in a partial vacuum and the results agreed, in about five-sixths of the cases, with the basal metabolic rate of the patients. These investigators believed that the test is of value and that more work should be done with this method.

Previous to the work on mountain sickness and the application of the

lack of oxygen as a test for thyroid activity, Mansfeld and Müller had investigated the stimulating effect of lack of oxygen on the thyroid. Lack of oxygen was believed to stimulate the thyroid gland since it brought about an increased elimination of nitrogen. When normal rats were held for a short time in an atmosphere of nitrogen, containing 7 per cent of oxygen, there was an increase in protein breakdown. Furthermore, the normal animal showed an increase in nitrogen output after severe hemorrhage and after the injection of small amounts of hydrocyanic acid. These authors concluded that hydrocyanic acid, lack of oxygen and hemorrhage all result in stimulation of the thyroid gland. The removal of the thyroid from the experimental animal prevented the increased output of nitrogen, and it was further shown that if thyroid was given to thyroidectomized animals, the administration of hydrocyanic acid, the production of lack of oxygen or a severe hemorrhage did not result in an increase in the elimination of nitrogen. They suggested that the thyroid cells are far more sensitive to anoxemia than the other cells of the organism; consequently when anoxemia is once produced, even though of slight degree, the thyroid function is increased as an immediate result. This causes an increased sensitiveness of the animal to lack of oxygen and so a vicious circle is established.

Yosomiya has recently investigated some other physiological factors regarding the influence of lack of oxygen. He showed that animals which were fed with thyroid material had a greater consumption of oxygen than normal and were therefore more sensitive to lack of oxygen. After administration of glucose the duration of life, in an atmosphere deficient in oxygen, was prolonged. Although the intake of oxygen was not affected, the insufficiency of oxygen compatible with life might be increased to a greater extent after carbohydrate was administered to the animal. If insulin was administered the tolerance of the animal to lack of oxygen was decreased and the duration of life in a low concentration of oxygen was shortened. After the injection of insulin the deficiency of oxygen in the arterial blood was reached relatively quickly and the carbon dioxide was increased.

In the hypothyroid state the maximal deficiency of oxygen which was compatible with life and the duration of the animal's life in the condition of anoxemia were both increased. Oxygen deficiency and the carbon dioxide content of arterial blood decreased more slowly after the administration of glucose in such an animal, but both oxygen deficiency and high carbon dioxide content were increased after the administration of insulin.

The observations concerning the sensitiveness of the animal organism to lack of oxygen are in keeping with the hypothesis that the thyroid gland is intimately involved in processes of oxidation. The fact that these reactions are also markedly influenced by the presence of carbohydrate and insulin constitutes still more evidence that the physiological effects of the thyroid are intimately bound up with the action of other ductless glands and are peculiarly dependent on the physical state and the metabolites which are present in the tissues of the body.

Whether or not the test on the rat will prove to be of clinical value appears questionable: it depends on many variables. It seems highly probable that the injection of relatively large amounts of serum on three successive days must of itself modify the condition of the animal to such an extent that interpretation of the response to subsequent lack of oxygen is difficult. There can be no question that both the administration of thyroid material and thyroidectomy produce marked changes in the sensitiveness of the animal to lack of oxygen. This is a most important and significant observation.

The questions whether the thyroid gland is itself sensitive to anoxemia and whether the increased nitrogen output after a period of anoxemia is actually due to an increased rate of discharge of the thyroid secretion are important aspects of the problem concerning the stimulation of the thyroid gland. It is hoped that the investigation of this physiological problem will be continued and that the activity of the thyroid will be established by determination of the basal metabolic rate as well as by the increase in nitrogen metabolism.

SUMMARY

The normal animal organism is susceptible to a diminished concentration of oxygen. If the thyroid gland is removed an experimental animal can then tolerate a lower concentration of oxygen. Administration of thyroid material increases the susceptibility of an animal to lack of oxygen. This test has been used for a biological assay of thyroid material. The injection of glucose has been shown to increase both the duration of life in an atmosphere deficient in oxygen, and the insufficiency of oxygen compatible with life. The injection of insulin produces the opposite effect.

Chapter 12

The Thyroid and Carbohydrate Metabolism

Variations in the carbohydrate metabolism in relation to thyroid activity have been observed in man and animals. Investigations of this phase of the thyroid have been concerned with, first, the effect of the removal of the thyroid and parathyroid; second, the content of glycogen in the liver; third, hyperglycemia, and fourth, glycosuria. Even before the use of thyroid extract for the relief of myxedema had been reported and several years before iodothyrin had been separated from the gland, Falkenberg studied the influence of the extirpation of the thyroid on sugar metabolism. The thyroids and parathyroids of twenty dogs were removed; in thirteen, sugar was present in the urine; sixteen dogs died, eleven of which showed sugar in the urine. Of the four dogs that lived, two dogs manifested glycosuria. The presence of sugar was not transient, but persisted in one of the dogs for three weeks.

Hirsch and Underhill and Saiki have investigated the influence of the removal of the thyroid on blood sugar and the glycogen content of the liver. Underhill and Blatherwick found that the glycogen entirely disappeared from the liver and the blood sugar was markedly lowered after thyreo-parathyroidectomy.

Parhon [394] reported that following thyroid-parathyroidectomy of dogs the liver and muscles contained less glycogen. The normal liver content was 2.87 per cent and the glycogen in the liver following thyroidectomy was reduced to 0.215 per cent. The glycogen content of the muscles was reduced to one-third the normal figure.

Eppinger, Falta and Rüdinger [144, 145] reported that after removal of the thyroid gland epinephrine injections did not lead to glycosuria even when glucose was administered. They suggested that the thyroid, parathyroid and pancreas are all involved in the regulation of carbohydrate and protein metabolism.

One variable factor in the early investigations on the relation between the thyroid apparatus and carbohydrate metabolism was the functional capacity of the parathyroid gland after thyroidectomy. The later re-

searches, particularly since the separation of the active agent from the parathyroid, have emphasized the importance of the parathyroid glands in carbohydrate metabolism.

THE INFLUENCE OF THE ADMINISTRATION OF THYROID MATERIAL ON LIVER GLYCOGEN

Absence of thyroid activity has been shown to affect markedly the storage of glycogen in the liver.

Parhon [393] showed that the administration of thyroid produced the same effect. The glycogen content of the liver of rabbits was much decreased after the administration of thyroid material although the glycogen content of muscle was but slightly below normal. The suggestion was made that the thyroid increased the glycemic function of the liver and transformed the glycogen into glucose although the respiratory exchange was but slightly increased.

Herring confirmed the observation of Parhon that glycogen disappears from the liver after medication with thyroid material.

Kuriyama [300] found that thyroid administration caused glycogen to leave the liver; however, two to three days after the cessation of thyroid medication the liver glycogen was again normal. He further found that glucose was not rapidly converted into glycogen in the liver of rats which were in the hyperthyroid state, although this occurred rapidly in fasting rats in which the glycogen content of the liver was low.

The influence of thyroid material on glycogen in the liver was extended to a study of thyroxine by Romeis.[427] The administration of thyroxine to mice brought about a complete disappearance of glycogen from the liver. He confirmed my observations that several small doses of thyroxine gave a more marked result than one large dose. He showed that 0.057 mg. of thyroxine distinctly reduced the liver glycogen and twice this amount caused its complete disappearance. There were marked differences in the sensitivity to thyroxine which probably depended on variations in the age, sex and weight.

Abelin, Goldener and Kobori showed that in rats which were fed thyroid material glycogen was not formed from the carbohydrate, but, if before or during the feeding with thyroid fat was given, the liver was able to transform carbohydrate into glycogen. Yolk of egg, cod-liver oil and the higher fatty acids had the same effect. Brain matter rich in lipoid was ineffective. They suggested that the glycogen in the liver of animals which were fed fat during the administration of thyroid material, was derived from carbohydrate and not fat. It appeared probable

that during the administration of thyroid material glycogen was formed, but was immediately removed from its deposits. In order to show the difference in the glycogen-storing power of the liver during the administration of thyroid and epinephrine a series of animals were injected with epinephrine on several successive days; on the last day cane sugar was given. Seven hours later the animals were killed. The glycogen content of the liver was very high. The maximal amount found was 6.87 per cent. This was evidence that the effect of epinephrine on lycogen metabolism was entirely different from the effect of thyroxine.

Cori and Cori have recently confirmed the observation that the effects of thyroxine and epinephrine on liver glycogen are not at all similar. These authors have shown that the injection of epinephrine decreased the glycogen in the muscle and at the same time increased the glycogen content of the liver. They suggested that the mechanism might be a conversion of the muscle glycogen into lactic acid and a reconversion of lactic acid into glycogen in the liver.

Cramer and M'Call found that removal of the thyroid and parathyroid from rats did not severely disturb metabolic processes. There was no impairment in the power to oxidize carbohydrates. From this fact it was inferred that the condition of carbohydrate metabolism in experimental hyperthyroidism was not due to a direct stimulating action of the thyroid active agent on the oxidation of carbohydrates. Carbohydrate metabolism appeared to be influenced by a mobilization of liver glycogen which was followed by increased oxidation. This fact implied that the organism responded to a discharge of sugar into the system by oxidation of the sugar. The mobilization of liver glycogen led to increased oxidation of carbohydrates and not to glycosuria.

The influence of the thyroid on carbohydrate metabolism was still further investigated by Cramer and Krause who found that after the administration of fresh thyroid gland for two or three days to rats or cats which were at the same time fed on a carbohydrate-rich diet, the liver contained only traces of glycogen. They concluded that the influence of the thyroid inhibited glycogen formation as there was no increased utilization of carbohydrates, and thyroid administration was not accompanied by glycosuria. Other experiments on dogs showed that the tolerance for glucose was only slightly diminished by thyroid medication.

Cramer reviewed the subject of glycogen metabolism and suggested that the glycogenic function of the liver was inhibited by the pancreatic hormone and was stimulated by the sympathetic nervous system and by

the thyroid and suprarenal hormones. This stimulation and inhibition affected both the formation of carbohydrate by the liver and the secretion of glucose into the blood. The antagonism between these two groups of hormones was restricted to the glycogenic function of the liver and not to the carbohydrate metabolism of individual cells and organs. He pointed out that this must be true since both groups of hormones increased the consumption of carbohydrates by individual organs, although in different ways. The stimulation of the glycogenic function by the thyroid and suprarenal hormones led in a normal organism to increased oxidation of carbohydrate, increased formation of carbohydrates from proteins, increased heat production, and increased nitrogen excretion. This stimulation occurred in such conditions as experimental hyperthyroidism, exposure to cold and fever.

Sanger and Hun suggest that sugar, when offered to the tissues as a fuel, is burned with great avidity, in hyperthyroidism. The increased utilization of carbohydrate after its ingestion, along with the maintained high level of blood sugar, pointed toward an inability to store glucose, most probably a failure of liver storage, due to some toxic change in the liver caused by the disease.

Richardson, Levine and Du Bois have presented the result of an investigation which questioned the observations concerning the inability of the body to store glycogen. Studies of the heat production in two cases of exophthalmic goiter during starvation showed that the patients must have had as much glycogen available as a normal person. They suggested that the liver may store glycogen easily and use it easily. In one case acidosis developed, despite the combustion of glycogen, with a respiratory quotient, 0.75. This is the respiratory quotient at which acetone appears in other conditions. The danger of acidosis in hyperthyroidism was emphasized.

Kuriyama [301] has shown that a tendency to acidosis was produced by the administration of desiccated thyroid to rabbits. The carbon dioxide capacity of the plasma was greatly decreased. This effect, however, was influenced by the diet. Acidosis was less marked on a green diet than when the rabbits were given oats.

During the experimental hyperthyroidism there was but little change in the hydrogen-ion concentration of the blood and the acidosis disappeared a short time after the administration of thyroid material was stopped. Kuriyama suggested that the acidosis was probably due to increased production of acids in the body. Epinephrine failed to cause any noteworthy acidosis. When a small amount of sodium bicarbonate

was administered to rats that were in a hyperthyroid condition the decrease in the glycogen content of the liver was nearly as great as in animals that were not given the alkali.

The absence of glycogen in the liver after the administration of thyroid material, which has been shown by many investigators and the results of Richardson, Levine and Du Bois on the respiratory metabolism in hyperthyroidism, illustrate the difficulty of forming conclusions from experimental results which involve either a transient or prolonged hyperthyroidism. It is possible that during the acute stage induced by thyroid administration or in the early stages of hyperthyroidism in man, the amount and distribution of glycogen in the body is entirely different from that present in a long-continued state of hyperthyroidism.

THE EFFECT OF AMINES AND OTHER SUBSTANCES ON CARBOHYDRATE METABOLISM

Abelin and Jaffé have shown that phenylethyl and *para*-oxyphenylethylamine and tyramine not only increased the basal metabolism but that they also caused complete disappearance of glycogen from the liver. So far as the glycogen-storage power of the liver was concerned these three proteinogenous amines behaved in a manner similar to thyroid material.

Romeis [427] has confirmed the work concerning the effect of tyramine, phenylethyl and *para*-oxyphenylethylamine and has shown that di-iodotyrosine markedly reduced the liver glycogen. The amount of the amines which was required to bring about this effect was much greater than that of thyroxine.

Romeis and von Zwehl found that, although the glycogen in the liver of mice that had been fed di-iodotyrosine was reduced, the glycogen content of the liver of mice fed sodium iodide was normal. From five hundred to one thousand times as much di-iodotyrosine as thyroxine was required to bring about the same loss in body weight and decrease in glycogen content of the liver.

Kobori has investigated the effect of inorganic salts on the formation of iodine compounds in the thyroid and glycogen in the liver. He found that on a calcium-poor diet the thyroid gland was able to retain only little iodine, whereas on foods rich in potassium it stored larger amounts of iodine. The influence of these cations on sugar metabolism was also shown. The glycogen content of the liver was the largest with a diet rich in calcium and smallest on a potassium-poor diet.

THE INFLUENCE OF THE THYROID GLAND ON BLOOD SUGAR IN EXPERIMENTAL ANIMALS

The significance of a high content of blood sugar in experimental animals has been extensively investigated in relation to thyroid activity. Böe concluded that neither hyperthyroidism nor hypothyroidism produced changes in the blood sugar content in rabbits. He also reported that the thyroid did not change the action of epinephrine in its effect on blood sugar. Kuriyama [300] found that thyroid feeding did not change the blood sugar content in cats or rabbits and did not produce glycosuria.

Hancher, Hupper, Blau and Rogers found that a glycerol extract of fresh thyroid glands of the pig brought about an increase in blood sugar in the dog. Extracts from desiccated thyroid and other thyroid preparations did not cause a change in the blood sugar.

Riddle, Honneywell, and Spannuth have studied carbohydrate metabolism in a group of pigeons. Those birds with an abnormally low blood sugar content were found to have large thyroid and large suprarenal glands. Those with high blood sugar were found to have small thyroid and small suprarenal glands. These investigators suggest that both thyroid and suprarenal glands are in some way concerned in the establishment of the normal blood sugar level.

Bodansky investigated the effect of thyroxine on the blood sugar of sheep and found that blood sugar was increased after the administration of thyroxine although the effect was temporary. Larger increases in blood sugar were obtained, after a latent period, when thyroxine was administered every other day. After thyroidectomy the average blood sugar level was maintained only during the first weeks of treatment with thyroxine. Throughout this period the fasting blood sugar was increased, probably at the expense of the glycogen store which was present at the beginning of the experiment. The blood sugar content of the fasting cretin sheep, whose glycogen reserve had been presumably partly depleted, was low. The mean content of blood sugar in fasting normal sheep was raised by thyroxine, presumably at the expense of the glycogen store. The fluctuations in blood sugar were within a small range in untreated animals, but were much greater in those treated with thyroxine. It was difficult to estimate accurately the total effect of thyroxine on the blood sugar unless frequent determinations were made. Bodansky concluded that the cause of the fluctuations in blood sugar may be dietary.

Ducheneau showed that thyroidectomized rabbits were sensitive to the diminution in blood sugar content produced by the administration of

insulin. Britton and Myers have confirmed and extended these observations. Other investigations which show a relation between the thyroid and carbohydrate metabolism have been carried out by Abderhalden and Gellhorn,[2] Aszodi and Ernst, and by Burn and Marks. Burge and Williams showed that thyroxine prevented tetany after insulin administration and also retarded the utilization of sugar by Paramecium.

Shpiner found that the administration of thyroid to an animal with an intact pancreas did not produce any effect on the blood sugar, but that in a partially pancreatectomized dog hyperglycemia and glycosuria, associated with fever, were produced. On the basis of these experiments he concluded that there is no direct but an indirect relationship between the thyroid and the pancreas.

HYPERGLYCEMIA AND GLYCOSURIA ASSOCIATED WITH HYPERTHYROIDISM

Geyelin investigated forty cases of hyperthyroidism, and found that hyperglycemia was a common accompaniment of this condition. Thyroid extract, fed to patients with myxedema who previously showed normal blood sugar values, produced hyperglycemia. Janney and Isaacson found that thyroidectomy caused a decrease in the sugar content of the blood, and they state that there was a delay in the assimilation of glucose from the blood after thyroidectomy.

The relation between the thyroid and carbohydrate metabolism and the interrelationship of other ductless glands have been studied in great detail in hyperthyroidism. Glycosuria, either spontaneous or alimentary was frequently present after the administration of 100 grams of glucose in a large group of cases studied by Geyelin.

The relation of hyperthyroidism to diabetes, considered primarily from the viewpoint of an investigator interested in diabetes, has been well presented by Allen and by Wilder. Allen [21, 22] has expressed the opinion that excess of thyroid activity may aggravate the symptoms of an existing diabetes, but that it could contribute to the actual causation of diabetes had never been demonstrated. He attributed suppression of diabetic glycosuria and hyperglycemia in the condition of thyroid deficiency to injury or cachexia and did not believe that the intrinsic severity of diabetes is lessened by diminished thyroid activity; finally he suggested that neither the excess nor deficiency experiments can properly be interpreted in favor of an antagonism between the pancreas and the thyroid, or a thyroid element in diabetes.

Wilder has shown that diabetes is not frequently associated with hyperthyroidism (less than 2%) and that exophthalmic goiter is still

less frequently complicated by diabetes (less than 1%). A mild and inconspicuous diabetes might be markedly increased by hyperthyroidism and severe hyperthyroidism might provoke coma in a diabetic patient. He found that the requirement of insulin was increased by hyperthyroidism, and that thyroidectomy was almost always followed by a gain in sugar tolerance in diabetes. In some cases this was great enough to suggest an actual cure of the diabetes. Wilder reported a case of diabetes in which there was a palliative effect of myxedema. When the basal metabolic rate was restored to normal, by administration of thyroid, the diabetic condition was manifest. In hyperthyroid cases there was considerable danger associated with hypoglycemia after the use of insulin. He found some patients peculiarly sensitive to over-doses of insulin. The principal relationship between hyperthyroidism and diabetes was explained by the elevation of the metabolic rate which was induced by the hyperthyroidism. At a lower rate the tissue cell was able to utilize a given amount of glucose with less insulin than that which was required at a higher metabolic rate. If the metabolic rate was increased a disproportionally increased amount of insulin was required to control the diabetes.

Carbohydrate metabolism in hyperthyroidism is well presented in a recent article by John. From a study of a large group of cases, John concluded that there is no glucose tolerance curve specific for hyperthyroidism. A definitely diabetic type of curve may be present in a mild case of hyperthyroidism and a normal curve may be present in a very severe case of hyperthyroidism. Thyroidectomy improved the carbohydrate tolerance. John found that glycosuria and hyperglycemia are not uncommonly present in hyperthyroidism and he suggested that their significance and relationships to carbohydrate metabolism should be determined by appropriate tests. The glycogen depletion resulting from hyperthyroidism increased the tendency to acidosis: this tendency was aggravated in cases in which diabetes was present.

SUMMARY

The removal of the thyroid and parathyroid glands may produce glycosuria and bring about a disappearance of glycogen from the liver. The latter effect is produced by administration of thyroid material. Stimulation of the glycogenic function by thyroid material leads to oxidation of carbohydrate and not to glycosuria. The hormones of both the thyroid and suprarenal glands increase the consumption of carbohydrate. A normal glycogen supply which can furnish carbohydrate dur-

ing starvation has been found in some cases of exophthalmic goiter. Phenyl and oxyphenylethylamine and tyramine cause a complete disappearance of glycogen from the liver. Neither hyperthyroidism nor hypothyroidism produce constant changes in the blood-sugar content in rabbits or in sheep. Hyperglycemia and glycosuria are associated with hyperthyroidism: their significance to carbohydrate metabolism should be determined by appropriate tests. There does not appear to be a direct, but there is an indirect relationship between the thyroid and the pancreas.

Chapter 13

The Influence of the Thyroid Gland on Growth, on Certain Phases of Cellular Activity in the Animal Organism and on Unicellular Organisms

One of the most striking symptoms of hypothyroid function in early life is the lack of growth. The stimulus which normally causes the development of the long bones appears to be almost wholly lacking, and cretins, twenty, thirty and even fifty years of age, are seen with a body length of children of four and five years. It has been demonstrated many times that the administration of thyroid material to a thyroid-deficient child results in prompt stimulation of the growth of the long bones, and growth which is normal in all respects can be brought about even in a high-grade cretin if administration of thyroid material is started at an early age. Unless thyroid medication is started early in life secondary changes may occur. There are a few recorded cases in which thyroid administration, even after the age of ten, has brought about marked growth. In certain instances the effect was spectacular. There is the well-known case of the young British student who desired to enter a military college, but was unable to do so because he was too short. He consulted a physician, who suggested the use of desiccated thyroid. During the following six months this young man was able to grow seven inches (Gibson).

The ability to stimulate growth in the hypothyroid child has been demonstrated with thyroxine. A striking case was that of a child who was nine years old when brought to The Mayo Clinic, in 1914. She was 37 in. in height and weighed 37 pounds. From January, 1915, to January, 1916, thyroxine was administered daily and during this interval she grew 6 in. All other hypothyroid symptoms were relieved at the same time, but in no respect did she show more marked response than in the growth of the long bones. During the succeeding five years she continued to increase in height and weight (Table 14). The increase in

TABLE 14. *Increase in Weight and Height of a Cretin Treated with the Acid-Insoluble Products of Alkaline Hydrolysis of Thyroid Material (Kendall)* [271]

Date	Weight Pounds	Height Inches
January 4, 1915	37	37
January 27, 1915	35⅓	38
February 27, 1915	36¾	38
April 2, 1915	38	39
May 5, 1915	37	39
June 4, 1915	37½	39¾
July 8, 1915	39	41
August 5, 1915	41½	41½
September 4, 1915	41½	41¾
October 16, 1915	42¾	41¾
November 13, 1915	45	42
December 14, 1915	44¾	42¼
January 16, 1916	45½	43
February 15, 1916	44	43¼
March 17, 1916	45½	43¾
April ?, 1916	43	43¾
May ?, 1916	46	44
June 18, 1916	47	44⅛
July 14, 1916	46½	44½
August 16, 1916	47½	45¼
September 17, 1916	48½	45½
October 22, 1916	50	45½
November 17, 1916	50½	45¾
December 6, 1916	50½	45⅝
January 16, 1917	52	46
March 13, 1917	52	46½
April 19, 1917	53	46¾
May 25, 1917	54	46¾
June 19, 1917	53	47
July 23, 1917	53	47
August 22, 1917	53	47¼
September 25, 1917	54	47¾
October 29, 1917	56	47¾
November 27, 1917	57	48
December 20, 1917	56	48⅓
January 16, 1918	57	48⅓
February ?, 1918	58	48⅓
March 30, 1918	58½	48⅓
April 20, 1918	59	48⅓
May 30, 1918	58½	48½
June 26, 1918	58	48½
July 21, 1918	57½	48½
August 30, 1918	58	48½
September 24, 1918	57	48¾
October 28, 1918	58	49
November 30, 1918	58½	49½
December 31, 1918	58	49¾
January 31, 1919	59½	50¼
February 27, 1919	60	50¼
March 23, 1919	62	50¼
April 27, 1919	60	50½
June 22, 1919	59½	50½
July 21, 1919	60	52
August 29, 1919	65	52½
October 20, 1919	64	52½

TABLE 14.—*Continued*

Date	Weight Pounds	Height Inches
November 30, 1919	67	53
January 1, 1920	68	53¾
February 13, 1920	68½	54
April 3, 1920	70	54
Increase in weight	89 per cent	
Increase in height	46 per cent	

mental activity was striking; her mental age at ten years of age was not greater than that of a child of three. (See Figures 30 to 33 inclusive.)

FIG. 30.—A cretin before treatment was begun.

FIG. 31.—One year later, during which time thyroxine in partially purified form was given. Increase in height, 6 inches.

Many similar cases of marked increase in growth are recorded in the medical literature.

EFFECT OF THYROID ON GROWTH OF YOUNG RATS

Another phase of this problem has been extensively studied: the effect of the thyroid gland on the growth of young mammals.

Schäfer and Fordyce observed the effect of the administration of thyroid on rats. Hoskins [231] has summarized the earlier literature and

has shown that thyroid feeding in white rats produced a decided hypertrophy of the heart, spleen, liver, kidney and suprarenal glands, especially in the male. Herring fed thyroid material to young white rats and found that it tended to diminish body weight. He confirmed the results of Hoskins: thyroid feeding caused profound changes in many of the organs and led to fatal results. He observed enlargement of suprarenals, heart, liver, spleen, testes and ovaries. The pancreas was hypertrophied; the thyroid gland was decreased in weight and showed signs of relative

FIG. 32.—A cretin before treatment was begun.

FIG. 33.—Same patient as shown in Fig. 32 six months later. Increase in height, 4 inches.

inactivity. The heart and kidneys were sometimes more than tripled, the suprarenals and pancreas more than doubled in weight. There was increased production of epinephrine.

Cameron and Carmichael [90, 92, 93] have extended these earlier studies and have thoroughly investigated the influence on the growth of the young animals of not only whole thyroid gland, but also of thyroxine and other preparations made from the thyroid. This work showed that thyroid material retarded the growth of young animals, rats and rabbits, and that at the same time there was a hypertrophy of certain organs, the

liver, kidney, heart, pancreas and suprarenals. If the dose of thyroid material was sufficiently large there was definite retardation in the growth of the thyroid gland which appeared anemic and was apparently in a resting condition. These effects could be produced in the adult rat as well as in the young and were directly traceable to thyroxine. They were not produced by administration of iodides, parathyroid, or any of the other ductless glands, and they could not be attributed to a high-protein diet.

Hewitt first showed that the organ hypertrophy following administration of thyroid was only temporary and that after cessation of thyroid administration there was a return toward a normal condition. In a large series of animals Cameron and Carmichael [92] have confirmed the work of Hewitt and have shown that, although the growth rate was decreased during the administration of thyroid material after the cessation of such feeding, the rate of growth was accelerated above normal so that the weights of treated rats usually exceeded the weights of the controls four or five weeks after the cessation of thyroid feeding. Cameron and Carmichael concluded that the cause of this acceleration was associated with the organ hypertrophy which had been produced by the thyroid feeding. Not only was there marked hypertrophy of liver, kidney, heart and suprarenals, but furthermore muscle tissue was distinctly diminished; these findings suggested a status of hyperthyroidism in the rats. The results were explained by an abnormal tissue catabolism and at the same time an immediate oxidation of the circulating food units which were broken down in great part before utilization by the tissue cell. This increased activity of the tissues called forth hypertrophy of the essential organs of the body, heart, liver, kidney, pancreas and suprarenals, with the exception of the thyroid gland itself, which was found in a resting condition because of absence of stimulation to the gland. Administration of thyroid material, therefore, resulted in the production of an engine of higher power than normal and after cessation of the abnormal stimulus, growth processes proceeded at a rate above normal. However, after the stimulus on the essential organs was removed they gradually returned to their normal capacity. In a period of from four to seven weeks after cessation of thyroid feeding the distinct hypertrophy which was produced in eighteen days had almost if not quite disappeared. When Cameron and Carmichael [91] investigated the influence of thyroxine on the growth of young rats they found the effect of this substance to be identical with that of desiccated thyroid. They concluded that "thyroxine answers satisfactorily to all the tests to which

it can be subjected and is undoubtedly the essential chemical compound secreted by the thyroid gland."

In a still further study of the effect of feeding thyroid material to young rats, Cameron and Carmichael [93] showed that this substance administered to young normal animals may set up a thyroid imbalance which may itself lead to subsequent growth disturbance and organ hypertrophy; furthermore, it was shown that a second period of thyroid administration produced a more marked effect on growth than did the initial period. They believed that there may be a possible risk in feeding thyroid material to young animals, including children, whose thyroid glands are normal.

THE INFLUENCE OF THE THYROID ON THE BONES, SKIN AND HAIR

The influence of the thyroid gland on the growth and calcification of bones in experimental animals has been shown in many investigations. Terry has shown the effect of thyroidectomy in frogs. The removal of the thyroid gland greatly retarded, if it did not stop, both the process of ossification and the process of growth in the bones of the hind legs; while calcification of the cartilage proceeded in the absence of the thyroid gland there was an extreme retardation in the process of ossification. Bircher [44] has shown that the thyroid has an influence on both the calcification and growth of the bones of rats. Calcification and rickets have also been investigated by Bosañyi who found that the administration of epinephrine and pilocarpine induced healing in rickets presumably by stimulation of the bone marrow. Hemoglobin favored the healing of rickets in rats and children. The changes in the phosphorus content of blood depended upon the process of calcification.

Kunde and Williams have recently investigated the influence of the thyroid gland in relation to the control of experimental rickets. They have found that in cretin rats no amount of cod-liver oil added to a ricket-producing diet was adequate to prevent the development of rickets. Histological study of the epiphysis was used as a criterion. Antirachitic vitamines supplied in the form of vitamine-rich foodstuffs added to a nutritious diet consisting of table scraps did not prevent the occurrence of rickets.

Hoskins [233] has shown a most interesting influence which was produced by the administration of the mono-acetyl derivative of thyroxine. The rate of growth of bony structures in the new-born rat was accelerated. The animals were injected with 0.1 mg. of the acetyl of thyroxine every other day; they began to show accelerated development after

the second injection. The changes became increasingly marked up to the fifteenth day, and then gradually diminished. Alterations in the proportions of the skull offered a convenient measure of the degree of precocity. Acceleration of development was visible in the epiphysis and also in the hair, eyes, nails, and ears of the injected rats. Growth, as measured by body weight, was not increased. There was marked loss in weight which was explained by the toxicity of the substance. Hoskins believed that thyroxine contains elements which affect the rate of differentiation apart from the rate of energy production. The most important factor which determined the response of an animal to thyroxine acetyl was its age, rather than its phylogenetic position.

Another aspect of the effect of the thyroid on the bones is shown by the changes in bone marrow. Lim, Sarkar and Brown showed that thyroid administration brought about an apparent increase in the activity of the bone marrow. This is in keeping with the observation that in myxedema there may be a secondary anemia, and it has been clearly shown that administration of desiccated thyroid and thyroxine in cases of hypothyroidism is followed by an increase in the hemoglobin and red-cell content of the blood.

Nakao [364] investigated the relation between the thyroid, thymus and spleen and the bone marrow. He showed that injection of sodium nucleate, after removal of the thyroid and thymus glands, did not change the blood cell count as it did before removal of the glands. He concluded that the absence of thyroid and thymus decreased the activity of the bone marrow, but (from other experiments) that the absence of the spleen alone did not decrease the activity of the bone marrow. In reporting another investigation,[365] he suggested that there is an interrelationship between the thyroid gland, thymus and the spleen and the blood-forming centers. The spleen apparently regulated the activity of the thyroid and the thymus in relation to the bone marrow in an inhibitory sense.

Thyroid and parathyroid deficiencies have been shown by Hammett [195] to produce changes in the bone during growth in respect to the water, ash and organic matter. Both types of glandular deficiency caused retarded ossification. Thyroid-parathyroidectomy brought about a lower calcium and phosphorus and a higher magnesium content in bones. Hammett [194] believed that the retardation in the growth of bones was largely determined by the relation of the specific type of growth metabolism of the bone to that of the body as a whole. The response of the bone to the lower metabolic or nutritional level produced by the removal

of the thyroid gland was of greater significance than any specific relation of thyroid function as such. The basic factors which determine systemic differential development are, according to Hammett, largely independent of the influence of the thyroid gland.

Hammett [196] has carried out an extensive investigation concerning the influence of removal of the thyroid gland on the growth of rats. In an article which summarized the work he concluded that distortion of differential development followed removal of either the thyroid or parathyroid gland except in the hypophysis, the submaxillary gland, and possibly the spleen. This was not due to any specific relation of glandular functions to growth of organs, but to the general metabolic disturbance induced by the deficiencies. The growth of some organs was more resistant and that of others was more sensitive than that of the body as a whole. The basis of this differential reaction was the relation of the metabolic processes of the organs in question to the body economy as a whole. The distortion was consistent in direction and produced a distinctly modified type of structural organization. Hammett feels that the changes induced by the thyroid have certain applications in evolutionary development.

Simpson [454] studied the condition of hypothyroidism in a large series of thyroidectomized sheep and goats. After thyroidectomy was performed on lambs there was retarded growth. The weight of the control lamb was in some cases as much as three times that of the cretin. If thyroidectomy was delayed until the age of three or four months, retardation was only slight. He noted that a difference in the rate of growth could be detected on the nineteenth day after thyroidectomy. He [455] showed that thyroidectomy in sheep had a marked effect on the cutaneous system. In certain cases there was loss of all wool from patches of the skin and in all cases a diminution in the weight of the fleece. The rate of horn growth was markedly retarded. This was apparently not due to a secondary effect induced by the gonads, but was brought about directly by loss of thyroid function. A condition was produced in the sheep comparable to myxedema in man.

One of the most constant clinical observations associated with hyperthyroidism and hypothyroidism in man is the soft, moist skin and heavy, luxuriant hair in the former and the coarse, dry, brittle, thin hair in the latter condition. Hsi Chun Chang has investigated the influence of the thyroid gland on hair growth. Starvation and thyroid feeding brought about changes in the hair of the albino rat.

The most striking relation between the thyroid gland and the skin was demonstrated in the so-called hairless new-born of animals with thyroid involvement. Welch, and Hart and Steenbock have shown that

FIG. 34.—New-born of sheep with goiter. Photograph taken by Dr. Howard Welch.

the condition in hairless pigs and sheep which are sometimes born in goiterous regions is due to thyroid disturbance with apparently a deficiency in the thyroxine content of the thyroid secretion. This condition

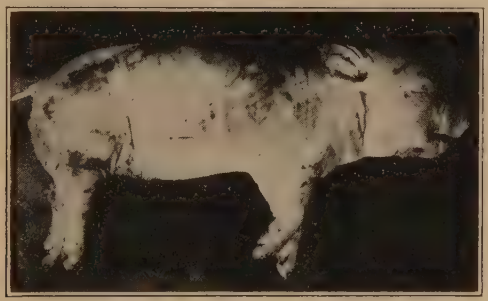


FIG. 35.—Hairless pig. This condition results in a high mortality rate, due to iodine deficiency. Photograph taken by Dr. Howard Welch.

is entirely relieved by the administration of iodine alone or by a change in the type of diet. (See Figs. 34 and 35.)

INFLUENCE ON THE PLUMAGE OF BIRDS

Closely related to the changes in the skin, hair and growth of horns are the alterations in the plumage of fowls. Torrey and Horning produced changes in the plumage of chickens by feeding thyroid material. At about the same time Parhon and Fils, and Parhon and Parhon observed retardation of growth and change in the appearance of the plu-

mage of young ducks after the administration of thyroid substance. Thyroidectomy influenced the development of the plumage of geese besides retarding the body growth. The result was a characteristic experimental infantilism. Cole and Reid showed that desiccated thyroid profoundly modified the appearance of the feathers of brown Leghorn fowls which were grown while the material was administered. It seemed probable that the thyroid material acted directly on the metabolism of the developing feather germ. The feeding of iodine, free or in inorganic combination, did not affect the plumage. Torrey and Horning [494, 495] showed that the administration of thyroid material tended to produce hen feathering in the Rhode Island Red male. The plumage appeared precociously but differentiated later than usual: other changes in the appearance of the plumage were present. They further observed the changes in the plumage of the domestic fowl produced by thyroid material. There was an acceleration in the eruption and differentiation of the feathers which would seem to involve an acceleration of division processes in the cells of the feather germ.

Zavadovsky investigated the plumage of fowls in regard to thyroid activity and the possible interpretation of these changes in evolutionary processes. Chickens were found to moult seven to ten days after the administration of thyroid material. The new feathers were either white or pale. Hens reacted slightly to a single dose of 30 to 50 grams of desiccated thyroid; however, 4 grams daily for six days was found to be excessive. A weight of meat equal to that of the thyroid fed produced no effect on the plumage. Ten mg. of thyroxine brought about moulting and all of the morphogenetic reactions brought about by 5 to 10 grams of desiccated thyroid. Zavadovsky assumed that thyroxine reacted directly on the feathers of birds, and suggested that the thyroid gland is involved in the normal physiology of the wool of goats and the hair and skin in myxedema. He believed that moulting and gonad activity are related and that the accumulation of the thyroid active agent in the ovary and egg may alter evolutionary relationships.

In further work, Zavadovsky and Rochlin investigated the effect of the administration of thyroid to nine different types of birds. Most of these reacted as does the female chicken, but depigmentation was not observed. One species of bird responded by increased pigmentation. They found that the crow was very resistant even to thyroxine. One variety of crow did not moult. They suggest that the great resistance of the crow family to thyroid medication may be regarded as a biological provision for birds that live on carrion.

Krizenecky and Nevalonnyj fed thyroid material to pure-bred chickens until a hyperthyroid state was obtained. To others both thymus and thyroid glands were fed together. The thymus did not affect the hyperthyroid condition. The administration of thyroid material brought about moulting, depigmentation and partial albinism. The normal moults after the condition of hyperthyroidism had been produced were influenced so that moulting appeared only on some parts of the body. Administration of thymus did not affect this. After the experimental hyperthyroidism had passed the plumage returned to its normal condition. There was no permanent depigmentation or albinism.

EFFECT ON UNICELLULAR ORGANISMS

Other evidence of a relation between the thyroid and growth has been sought in the development of tissue *in vitro* and the response of unicellular organisms.

Uchida has investigated the influence of the plasma of thyroidectomized rabbits on tissue growth *in vitro*. Cells were found to grow less rapidly in plasma which was taken from an animal from which the thyroid and thymus glands had been removed. The removal of the thymus increased the effect of thyroidectomy. From these experiments it was concluded that there is some growth-promoting principle in the plasma which comes from the thyroid and thymus glands.

Closely related to the experiments on tissue growth *in vitro* are those which have been carried out on a unicellular organism, Paramecium. In 1917, Shumway suggested that thyroid substance increased the rate of division as much as 65 per cent in *Paramecium aurelia* or *caudatum*. He also found disturbances of the excretory system. The most important of these was an increase of from two to three contractile vacuoles.

Abderhalden and Schiffmann also studied the division rate of Paramecium with extracts of many organs and concluded that an increased rate of division was brought about by an extract of the thyroid.

Cori found that a faintly alkaline solution of thyroid extract strongly accelerated the multiplication rate of Paramecium. With thyroxine he did not find any increase in the rate of division and concluded that the acceleration need not be due to a specific action of the thyroid active agent.

Torrey, Riddle and Brodie found that in solutions approximately of the same hydrogen-ion concentration as blood, thyroxine appeared to act like the total thyroid gland in the acceleration of certain metabolic processes in the Paramecium. On the other hand, unlike the total thyroid,

thyroxine depressed the division rate of Paramecium, the degree of depression varying with its concentration. The depression appeared to be due to the action of thyroxine directly on the Paramecium.

These results were confirmed by Woodruff and Swingle who suggest that the question of the measurement of the stimulation of cell division in Paramecium depends on the controls. The thyroid gland and other glandular material is a source of supply of more food for Paramecium. On the other hand, by the use of thyroxine the increased food supply is eliminated and a depression in the division rate is obtained. They believed that neither the thyroid, pineal, nor pituitary gland possesses intrinsic properties which accelerate cell division in the Paramecium; thyroxine does not accelerate the multiplication rate and above certain concentrations it actually depresses the rate of cell division in the Paramecium.

SUMMARY

The loss of thyroid function in early life is followed by a lack of development. Administration of thyroid to a cretin, even after the age of ten, may result in stimulation of growth. If marked secondary changes have occurred administration of thyroid is without effect on the growth. Thyroid administered to young rats brings about a retardation in the growth and marked hypertrophy of many of the organs of the body; after cessation of the abnormal stimulus growth processes proceed at a rate above normal. The hypertrophied organs gradually return to their normal capacity.

There appears to be a direct action of thyroxine on the growth of the bones. Thyroidectomized rats develop rickets: this condition is not relieved by the administration of antirachitic vitamines. The acetyl of thyroxine affects the growth of the bones in new-born rats: there is an effect on the rate of differential changes distinct from the influence on the rate of energy production.

There is an interrelationship between the thyroid and the blood-forming centers. Distortion of differential development of many organs follows removal of the thyroid. The thyroid has an effect on the cutaneous system; in thyroidectomized sheep there may be a loss of wool from patches of the skin. The rate of horn growth is retarded. Administration of thyroid produces changes in the hair of the albino rat. The most striking relation between the thyroid and the skin and hair is shown in hairless new-born pigs and sheep. In birds, moulting and depigmentation follow the administration of thyroid material.

Although desiccated thyroid increases the rate of division of the

unicellular organism, Paramecium, this is not due to the thyroxine content, but is due to the increased food supply furnished in the form of desiccated thyroid. Thyroxine does not accelerate the multiplication rate and it may actually depress the rate of cell division of Paramecium.

Chapter 14

The Influence of the Thyroid on the Metamorphosis of Amphibian Larvae

The physiological and clinical investigations of the thyroid gland between 1890 and 1912 closely linked oxidative processes in the animal organism and the gland. It was recognized that variations in thyroid activity influence the phenomena of growth and the development and condition of the hair, skin and bones. However, it was not until 1912 that Gudernatsch [185] applied this knowledge to a study of the differentiation and growth of the larval forms of amphibia. At that time he demonstrated the effect of the administration of various glands to tadpoles, and showed that by far the most active gland was the thyroid. The rate of metamorphosis was greatly accelerated and the time required to bring about the adult type of cell structure was reduced from many months to a few days. Gudernatsch's work was soon followed by many investigations concerning the effect of the thyroid gland and many iodine-containing compounds on the metamorphosis of various species of amphibia.

Morse showed that iodized blood albumin reacted in the same way as thyroid material. The iodine contained in the algae and colloidal iodine was not as effective.

Lenhart, Romeis,[424] and Rogoff proposed the effect of thyroid on the rate of metamorphosis of the tadpole as a test for the presence of thyroid material. Rogoff and Marine tested the products of alkaline hydrolysis of the thyroid gland, which I had suggested, and showed that the acid-insoluble fraction brought about the metamorphosis of tadpoles, but that the acid-soluble fraction was inactive. Romeis [426] showed that the acid-insoluble products after hydrolysis with barium hydroxide contained iodine and strongly affected the rate of metamorphosis of the tadpole.

Swingle [477] carried out an investigation concerning the acceleration of the metamorphosis of frog larvae in which he compared the changes in the various organs. He showed that the alimentary tract was the first

to be affected and pointed out that entire organs and systems of organs are transformed with startling rapidity from a larval condition to that characteristic of the adult, and yet in the midst of such somatic changes the gonads and germ cells remained unchanged.

The influence of various glands on the metamorphosis of the tadpole has been demonstrated. In 1916, Smith [458] successfully removed the hypophysis and showed striking changes in the other endocrine glands. In these larvae the thyroid gland was one-third its normal size and the tadpoles were albinos, which grew to unusually large size and did not metamorphose. Allen [20] and Adler [14] likewise removed the hypophysis and showed that it produced changes in the thyroid.

Swingle [483] agreed with Smith that the thyroid and pituitary glands are both necessary to the phenomenon of metamorphosis. A physiologically active thyroid gland transplanted into pituitaryless tadpoles initiated, but did not complete, the metamorphosis. The pituitary gland transplanted into thyroidless tadpoles produced no effect. The rate of liberation of the essential agent from the thyroid appeared to be controlled by the active constituent of the pituitary gland, since removal of the pituitary caused the thyroid gland to remain rudimentary and inactive.

Larson suggested that the pituitary can substitute for the action of the thyroid gland in maintaining the basal metabolic rate, and that there is a close relationship between the pituitary and the thyroid glands. He believed that the pituitary may function vicariously but that the thyroid cannot take the place of the pituitary.

Allen [19] was the first to remove the thyroid gland from *Rana pipiens* and to study the influence of its absence on the rate of metamorphosis. The absence of the thyroid gland did not affect the course of early development, up to the time when the hind legs began to grow. Further differentiation of the soma then ceased and metamorphosis did not occur. The administration of thyroid material brought about a resumption of growth and development even after four months.

Hoskins and Hoskins showed that the removal of the thyroid gland from amphibian larvae hastened growth and brought about hyperplasia of the hypophysis, but it prevented metamorphosis. Removal of the hypophysis retarded growth and development of the thyroid gland and also prevented metamorphosis. Feeding of thyroid gland or hypophysis hastened metamorphosis in the normal and thyroidectomized larvae. It was subsequently shown that this sample of hypophysis material contained iodine. Feeding hypophysis material to hypophysectomized

larvae did not cause metamorphosis but the administration of iodine to larvae after the removal of both thyroid and hypophysis brought about metamorphosis.

Schulze showed that transplantation of the thyroid gland to larvae produced the same effect as feeding thyroid material. Swingle [483] transplanted thyroid glands of newly metamorphosed *Rana clamata* intraperitoneally into nearly full-sized tadpoles and showed that these glands brought about practically complete metamorphosis within a period of from fifteen to twenty days. Transplantation of the thyroid glands of tadpoles into tadpoles of the same age had no effect. Swingle concluded that the thyroid gland must exert its effect on the development of the limb anlagen and not on the formation of the limb buds. Thydroidectomized tadpoles developed the limb buds but these stayed undifferentiated.

Slowikowska also determined the influence of the age of the tadpole on the transplantation of the thyroid gland to other tadpoles of the same age. Provided metamorphosis had not begun, transplantation of the thyroid gland of one tadpole into another did not induce metamorphosis. If the gland of a partially metamorphosed tadpole was transplanted to a second tadpole of the same age the rate of metamorphosis was accelerated. This is evidence that one reason for the delay in metamorphosis in the larvae of frogs must be the failure to elaborate the iodine-containing compound of the thyroid completely in the early stages of life.

The striking effect of thyroid preparations on the rate of metamorphosis of amphibian larvae instigated a large amount of research concerning the mechanism involved. Gudernatsch [186] had shown that tadpoles fed exclusively on thymus gland did not metamorphose. Uhlenhuth [504] later pointed out that the inhibitory effect of the thymus was not due to the presence of an antagonistic substance but to the absence of some substance required to develop the thyroid gland to a secretory condition. Uhlenhuth [505] further suggested that two substances were involved in amphibian metamorphosis: the first was iodine and the second was an excretor substance which acted on the thyroid gland and caused it to excrete its essential iodine compound. At low temperatures less excretor substance was produced than at a high temperature during equal rate of growth of tadpoles; therefore, larvae which were kept at a low temperature reached a larger size before they metamorphosed than those kept at a high temperature. Uhlenhuth [506] regarded the phenomenon of metamorphosis as controlled and dependent on a large number of individual factors. Instead of being regulated and kept in harmony by

the individual as a whole, the development of at least six groups of organs was controlled separately by the action of probably six different chemical mechanisms, each of which could be stopped or enforced independently.

As the mechanism of metamorphosis was further investigated it became evident that a large number of factors affect the physiological processes involved. Abelin [12] showed that phosphate salts influenced the action of the thyroid gland on the rate of metamorphosis. He found that when disodium phosphate was present the action of the thyroid gland fed to tadpoles was largely inhibited. Lim suggested that the effect of the thyroid gland on metamorphosis may be summarized in general terms as an influence which hastens and exaggerates normal processes.

There are many different theories and conflicting opinions concerning many phases of the problem of metamorphosis, but all investigators agree that iodine or some iodine-containing compound is essential for the metamorphosis of amphibian larvae. Abelin and Jaffé [13] have shown that, if metamorphosis had begun, tyramine slightly increased the rate of the reduction of the larval parts. This effect was only produced at certain stages in the metamorphosis. No experiments are recorded in which tyramine alone has induced metamorphosis in a thyroidectomized tadpole. The experiments, in which the thyroid gland was removed, and the investigations on neoteny are uniform in significance: unless the larvae can obtain iodine in some form metamorphosis cannot be induced.

Swingle [478, 479, 480, 481] showed that although di-iodotyrosine greatly increased the rate of metamorphosis, dibromotyrosine had no effect. The addition of bromine to the water in which tadpoles were kept did not bring about increased metamorphosis similar to that produced by a suspension of iodine. Elemental iodine was found to be much more active than any of the other iodine compounds investigated. Iodoform was the next most active compound, and then followed potassium iodide. Potassium iodate had no effect on the rate of metamorphosis.

With axolotl, Uhlenhuth [507] showed that 0.03 mg. of iodine present as iodothyrin in a liter of water brought about metamorphosis in thirteen days. From 33 to 86 times this amount of inorganic iodine had no effect.

Romeis [428] showed that of all iodine-containing compounds which influence the rate of metamorphosis thyroxine was the most active. It was much more active than iodothyrin, iodothyreoglobulin or di-iodotyrosine. It brought about changes when present in concentrations of one part in a million to one part in five billion. For these experiments the tadpoles were left in 500 cc. of a solution of the thyroxine, one part

in one million to one part in ten million, for from twenty-four to forty-eight hours. Except for this treatment the tadpoles were kept in 1000 cc. of water. After two treatments in the thyroxine solution metamorphosis became evident. The same result was obtained in solutions of thyroxine which contained one part in fifty million to one part in one hundred million, after three or four treatments. If the tadpoles were kept in a thyroxine solution continually, a concentration of one part in five billion was effective. This amount of thyroxine is beyond chemical identification and Romeis suggests that his previous observations concerning a protein-free preparation of the thyroid gland may be explained by the presence of traces of thyroxine. He further suggests that the observations of Herzfeld and Klinger,[217] who prepared an alcoholic extract which they found effective on rats, might also be explained by the presence of small amounts of thyroxine.

A large amount of work has also been carried out concerning the action of other iodine compounds on the rate of metamorphosis. Abelin [11] showed that di-iodotyrosine and iodo-albacid increased the velocity of metamorphosis. However, the mere presence of any iodine compound was not sufficient. Di-iodosalicylic acid, di-iodosalol, iodopyrin, the potassium salt of iodophenol sulphonic acid, di-iodothymol and iodogallicin were all inactive. Other glands and iodine compounds other than thyroxine could cause an increase in the velocity and type of metamorphosis, but they did not bring about the typical response which was induced by thyroxine and its derivatives.

Di-iodotyramine, di-iodotyrosine and iodo-albacid, Abelin suggests, are particularly to be considered if decomposition products of the thyroid are to be used instead of the whole thyroid substance. Abelin showed that di-iodotyrosine reacted in the same manner as thyroid material on axolotl as well as on tadpoles.

An interesting comparison of compounds closely related to thyroxine has been made by Gaddum, who has recently shown that synthetic thyroxine had the same effect on tadpoles as the natural thyroxine and that thyroxine was forty times more active than "di-iodothyroxine," that is the synthetic product with two atoms of iodine on the inner benzene ring and no iodine on the outer ring. Di-iodotyrosine was shown to have less activity than the same concentration of potassium iodide and less than one thousandth that of thyroxine.

Experiments on the metamorphosis of neotenous amphibians have shown that, although the axolotl which exists in the larval state for a long period can be made to metamorphose, other amphibian larvae per-

manently lose the ability to transform under the stimulus of thyroid material.

Swingle [482] failed to produce a change in the adult Necturus. These were fed, injected and grafted with thyroid glands, which were physiologically active, but with negative results. Twenty mg. of thyroxine had no effect, whereas a solution of one part in fifty thousand of this compound rapidly metamorphosed the immature larvae of Amblystoma. The perennibranchiate Necturus possesses a thyroid gland of great physiological activity as shown by heteroplastic transplantation into immature anuran larvae. The failure of the axolotl to metamorphose appears to be due to the inhibition or the defective development of some unknown factor which normally serves to release the thyroid secretion into the blood stream. In neotenous anuran tadpoles the delayed metamorphosis is apparently due to failure to secrete an essential substance because of some unknown inhibiting influence.

Swingle [483] believed that neoteny, both in urodeles and anurans, is due to inhibition of the secretory (excretory) functions of the thyroid gland rather than to any morphological defect of glandular structure, or inability to synthesize the physiologically active hormone. In contrast to this conclusion, Jensen found that the thyroid gland of the axolotl was in a pathological condition. In histological studies he found that the colloid material did not take the characteristic stain. In the young animal the cells lining the acini were cylindrical, but at three to six months of age pathological alterations became manifest.

Huxley also investigated the metamorphosis of axolotl and other amphibian larval forms. He concluded that different tissues are not uniform in their response and that there are variations in different species.

McCord and Marinus showed that irradiation with x-rays was without apparent effect on normal tadpoles, and that it brought about a slight increase in the susceptibility to thyroid stimulation.

THE RELATION BETWEEN METABOLIC RATE AND METAMORPHOSIS

The large number and scope of the experiments on the dependence of metamorphosis on the presence of iodine leave little question concerning this phase of the problem. Many other factors, however, have not been definitely determined. One attractive hypothesis is that metamorphosis is due to a change in the rate of the metabolism in the tissues. Provided the action of thyroxine is the same in all types of animal organisms it is obvious that administration of thyroid material will bring

about an increased basal metabolic rate; this explanation of metamorphosis, however, cannot apply to iodine itself or to di-iodotyrosine and the many other compounds which have no effect on the rate of metabolism, particularly when administered to thyroidectomized tadpoles. If iodine did increase the metabolic rate it would have to be through its effect on the thyroid gland and not through its action directly on the larval tissue.

It occurred to me that further light could be thrown on this subject by a comparison of the action of thyroxine and that of the acetyl of thyroxine. It was first shown (Kendall [271]) that the acetyl of thyroxine did not change the basal metabolic rate of a patient with myxedema and did not relieve any of the symptoms of hypothyroidism. It did not induce the typical effects of hyperthyroidism when administered to a normal dog. If, then, it could be shown that the acetyl of thyroxine brought about a change in the velocity of metamorphosis, this would be evidence that the effect was not due primarily to a change in basal metabolism. Experiments were carried out on the giant tadpole; thyroxine and the acetyl derivative were injected into the peritoneal cavity and the exact amounts which were required to bring about metamorphosis were determined. It became evident that the acetyl of thyroxine was but slightly less active than thyroxine itself, but it was far less toxic. The tadpoles which were injected with thyroxine lived but a short time after metamorphosis, whereas those injected with the acetyl did not die: they developed into miniature frogs.

Two conclusions were drawn from this work: the change in the velocity of metamorphosis was not due primarily to a change in the basal metabolism of the animal, and iodine could be furnished to the organism in the form which is required for an effect on the rate of metamorphosis by both thyroxine and the acetyl of thyroxine. The suggestion was made that the chemical reaction which induces metamorphosis is the production of hypo-iodous acid: those compounds which decompose with the production of hypo-iodous acid within the animal organism induce metamorphosis; those iodine compounds which do not, have no effect.

Swingle [484] questioned the validity of the tadpole test as a criterion for thyroid activity and emphasized my suggestion that there are two distinct effects of thyroxine: one on the basal metabolism and the other on the rate of metamorphosis. These two physiological effects involve two different chemical reactions.

Swingle, Helff and Zwemer repeated my work on the influence of

thyroxine and the acetyl of thyroxine on metamorphosis. They also compared the physiological response of these two substances, and showed that thyroxine produced the typical hyperthyroid effects, whereas the acetyl of thyroxine had no influence on the normal human being. With the tadpole, however, thyroxine and the acetyl both brought about metamorphosis. They concluded that the metabolic response and the amphibian response could not be compared and that they were due to two different chemical reactions induced by the thyroxine molecule.

Several investigators have determined the changes in basal metabolism during the various stages of metamorphosis. Joel determined the influence of temperature on the oxygen consumption of tadpoles before and after the ingestion of thyroid material. Twenty-six-day-old tadpoles consumed the largest amount of oxygen when the temperature of the water in which they were kept was 27°. The total oxygen consumed was 45 cc. for each 100 grams of body weight. As the temperature was increased the consumption of oxygen decreased to 35 cc. at 29.5°, and rose again to 45 cc. at 34°. The tadpoles died at a temperature between 36° and 37°. When this experiment was tried on frogs, recently metamorphosed through the action of thyroid material, it was shown that there was a maximum in the consumption of oxygen at 27°. This amounted to 89 cc. for 100 grams of body weight. Following the maximal rate of consumption of oxygen there was a sharp decline and the animals died at 33°. At all temperatures the oxygen consumed by the frogs was about twice that used by the tadpoles when compared on the basis of 100 grams of fresh tissue, but it was shown that the dry weight of frog tissue was equivalent to four times the dry weight of larvæ.

Groebbels pointed out that the oxygen requirements of the larvæ and frogs, which had been found by Joel, could not be compared, as the frogs which had received thyroid material were in a condition of hyperthyroidism. He found that the oxygen consumption of the larvæ was three times that of the normal frog when compared on an equal weight of tissue.

Groebbels used tadpoles in which the hind legs were developed, but the fore legs were not developed. He showed that the basal metabolic rate of such tadpoles was influenced to a marked extent by the diet. He assigned 100 to the oxygen requirement for 100 grams of tissue on the basal diet, 1. The relative oxygen requirements for 100 grams of tissue on a poor diet, 2, was 116. On a poor diet to which thyroid was added, 4, the oxygen requirement was 173, and on the basal diet plus thyroid medication, 3, it was 235. The speed of metamorphosis was shown to

vary with the different diets in the order 2, 4, 3, 1. Groebbels concluded that the oxygen requirements of normal tadpoles, in spite of constantly increasing growth, remained absolutely (for each unit of body weight) and relatively (for each tadpole) rather constant. The normal tadpole, compared with adult frogs, required absolutely less, but relatively more, oxygen. He also found that large adult animals had an absolutely larger and relatively smaller metabolism than smaller animals. The metabolism in insufficiently nourished animals was relatively larger than in normal animals of similar age; whether this increase depended on the relative action of hunger or the effect of avitaminosis, or on both factors, was uncertain. Normally nourished animals, which had been fed thyroid material, had absolutely and relatively a greater oxygen requirement. With decrease in body weight the oxygen requirement increased absolutely and relatively.

Groebbels assumed that there was a destruction of protein as a result of pathologically increased processes of dissimilation. Insufficiently nourished animals which had been fed thyroid material had relatively lower oxygen requirements than normally nourished animals. In agreement with earlier experiments, he believed the explanation for this to be a change in the thyroid action as a result of insufficient food and that metamorphosis was a result of effects which expressed themselves in metabolism during an insufficiency of food and thyroid feeding. The work of Groebbels shows clearly that in studies of oxygen consumption with amphibian larvæ the diet must be controlled. This factor is as important in the metabolism of the tadpole as it is in mammalian forms of life.

Lenhart had previously pointed out that a high-carbohydrate diet decreased the rate of tail absorption and increased the length of life of tadpoles after the administration of thyroid. He also showed that without carbohydrate the rate of metamorphosis was increased and that temperature also had a marked influence.

Jarisch showed that by variations in the diet the course of development was changed. The protein-sparing power of food regulated the degree of regression of the larval parts after the administration of thyroid. Proper nutrition protected the larvæ from resorption of their larval forms. Jarisch concluded that the thyroid brought about its effect through its influence on metabolism. It increased metabolism and thus led to an accelerated resorption of the larval organs. This process (the resorption of larval organs) was the essential action in metamorphosis.

There was no necessity to assume a direct action which caused development independent of the indirect action of the thyroid on metabolism.

Jewell showed that changes in hydrogen-ion concentration brought about marked changes in the rate of metabolism, but did not cause metamorphosis: an increase in the concentration of acid brought about a decrease in the output of carbon dioxide.

Abelin and Scheinfinkel found that with *Rana esculenta,* there was an initial rise in carbon dioxide output after the administration of thyroid, with a subsequent decrease to a value which at the end of metamorphosis was from 50 to 70 per cent of the previous value. Di-iodotyrosine and di-iodotyramine brought about the same reaction. They also observed a similar effect on the axolotl: an initial rise followed by a decrease. The desiccated thyroid was fed to the tadpoles from two to eight times during the experiment and the last feeding was three days before the end of the experiment. In the experiments with the axolotl di-iodotyramine was given eleven times, desiccated thyroid five times, and Lugol's solution was given over sixty days in increasing amounts from 0.1 to 1 cc. daily. Lugol's solution did not change the carbon dioxide output and there was no metamorphosis. The diet was meat only. The suggestion was made that the decrease in basal metabolic rate was a defense against excessive oxidation of body substances.

Helff has pointed out that the decrease in the basal metabolic rate, observed by Abelin and Scheinfinkel, is probably due to the decrease in the weight of the tadpoles. This would amount to from 50 to 70 per cent or more, and, provided the same amount of carbon dioxide was given off, there would be actually an increase in the basal metabolic rate for each 100 grams of body substance. Helff found a progressive increase in oxygen consumption for each gram weight during the various stages of metamorphosis as compared with that of the normal larvæ. The larvæ of *Rana pipiens,* metamorphosed by di-iodotyrosine underwent a gradual reduction in weight which amounted to 57 per cent. Di-iodotyrosine was found to be very efficient, although it induced a type of metamorphosis somewhat different from the natural. The increase in oxygen consumption was both absolute, for each gram of weight, and relative, for each individual. There was an actual rise in metabolic rate: the increase was regarded as secondary to metamorphosis, and not as a causative factor.

Gayda found that the production of heat was markedly increased in toad tadpoles after the administration of thyroid material. The maxi-

mum was reached on about the sixth day. Drastich showed that the carbon dioxide production and oxygen intake diminished during the larval development of *Salamandra maculosa;* there was a sudden increase at the time of natural metamorphosis.

Bělehrádek and Huxley have recently investigated the oxygen consumption in the axolotl and have found that for each unit of body weight there was no immediate effect brought about by the intraperitoneal injection of an alkaline solution of desiccated thyroid. After a latent period of from eight to fourteen days there was an increase in the metabolic rate which occurred at the metamorphic crisis. The larval skin was shed at this time and there were a number of morphogenetic changes and a loss of body weight, which just preceded this. Metamorphosed Amblystoma did not show a latent period before a rise in basal metabolic rate after an injection of thyroid material. It responded almost immediately to the administration of thyroid. It was suggested that one of the important changes which occurs during the refractory period after an injection of thyroid material was a change in physiological equilibrium which permitted the animal to respond to internal stimuli such as the active agent of the thyroid by an increase in metabolism. This changed equilibrium was apparently permanently retained in Amblystoma.

The results of the investigations on the basal metabolism in which it has been shown that the hydrogen-ion concentration, the type of diet, whether carbohydrate or protein, and the time of response to thyroid material, all indicate that the physiological response in the amphibian larvæ is closely related to the response in mammalian organisms. That the reaction is influenced by still other factors has been shown by Lundberg,[315] who treated axolotl with thyroxine and then injected from 1 to 20 units of insulin every fourth or fifth day, to a total of about five doses. He found that the administration of insulin prevented the metamorphosis of the axolotl. Control animals which were given the same amounts of thyroxine showed definite signs of metamorphosis. He believed that there was an antagonism between the pancreas and thyroid glands. He[316] further investigated the effects of extracts of other organs in relation to the action of the thyroid on the metamorphosis of axolotl. Extracts from the ovary and testis could not prevent the metamorphosis induced by the injection of thyroxine. Ovary extract had no effect on the male, but was found to inhibit the thyroxine effect to a considerable extent in the female.

The extensive work which has been carried out with the amphibian

larvæ in relation to thyroid activity has shown that the reaction furnishes a valuable method of testing the physiological activity of products derived from the thyroid gland, but that the effect on the rate of metamorphosis cannot be regarded as specific for thyroid material. Although all iodine compounds will not bring about the reaction, there are a large group of substances which will hasten metamorphosis. The concentration required of all iodine compounds not derived from the thyroid appears to be much higher than that of thyroxine or iodothyreoglobulin.

From the results which have been obtained it cannot be assumed that the metamorphosis is due primarily to a change in the basal metabolic rate. Neither is it clearly shown that there is an increased basal metabolic rate in all forms of amphibia after metamorphosis.

It has been shown with mammals that excessive treatment with thyroid material brings about a decrease in the basal metabolism; furthermore, it has been clearly shown that the response is largely determined by the caloric intake. It would appear that sufficient care has not been exercised in the control of the diet in a large number of investigations: medication with thyroid material both in respect to the dose and the frequency of administration has not been carefully controlled, and, in almost all of the experiments in which thyroid material was used, variations in the basal metabolic rate may have been due to the production of hyperthyroidism.

On the other hand, it is difficult to explain an increase in basal metabolism which is brought about by administration of di-iodotyrosine: Hoffmann has recently shown that this substance does not produce any change in the basal metabolism. Since compounds such as di-iodotyrosine, di-iodotyramine, and iodized proteins hasten metamorphosis, the essential influence of the iodine compound must be some reaction other than an increase in the basal metabolism similar to that which is brought about by thyroxine.

Before definite information can be secured it will be necessary to carry out studies in the basal metabolism of thyroidectomized larvæ with iodine-containing compounds which have been proved not to affect the basal metabolism. If under these conditions there is an increase in the basal metabolism then it would appear that this increased rate of oxidation is intimately associated with the metamorphosis. If, however, the iodine compound can induce metamorphosis, and the animal then passes into a condition in which the basal metabolism is below normal, it would suggest that the thyroid gland of the animal is stimulated by the proc-

esses of metamorphosis and that in the adult tissues of the amphibian there is an increased basal metabolic rate because of the increased activity of the thyroid gland of the metamorphosed animal.

THE INFLUENCE OF THE ADMINISTRATION OF THYROID ON SILKWORMS

Terao and Wakamori investigated the influence of the administration of thyroid material on silkworms and found that it did not bring about a visible effect. Continued investigation, however, showed that the second generation of silkworms were affected. The pupæ, cocoons and moths were smaller, yet more eggs were laid, and the amount of silk was increased. They point out that Abderhalden, Romeis and von Dobkiewicz were unsuccessful in demonstrating any effect of thyroid on insects and suggest that this may have been due to the fact that the experiment was not sufficiently prolonged.

SUMMARY

Thyroid material markedly increases the rate of metamorphosis of amphibian larvæ. The pituitary gland is involved with the thyroid in this phenomenon. The thyroid of frogs will induce metamorphosis after it has been transplanted into amphibian larvæ: the thyroids of amphibian larvæ are biologically inactive. A large number of factors are involved in the process of metamorphosis. One constant factor is iodine. Bromine or extracts of other glands not containing iodine are without effect. Many iodine compounds, but not all, will hasten the rate of metamorphosis. Potassium iodate is without effect; elemental iodine, iodoform and potassium iodide are active in the order given. The larval forms of some amphibians have permanently lost the ability to transform under the stimulus of thyroid material.

The basal metabolism appears to increase at the time of metamorphosis. The rate of metamorphosis is markedly increased with diiodotyrosine. This compound does not affect the basal metabolism in experimental animals. The acetyl of thyroxine and thyroxine both induce metamorphosis. The acetyl of thyroxine does not increase basal metabolism. This suggests that the influence of thyroxine on the rate of metamorphosis is not due directly to its influence on the rate of energy production. Thyroid material does not produce an immediate effect on the silkworm. The pupæ, cocoons and moths of the second generation are smaller yet the amount of silk is increased.

Chapter 15

Standardization of Thyroid Material

The experimental evidence which has shown the relation of the active agent of the thyroid gland to physiological processes has demonstrated its influence on the following: the relief of the symptoms associated with hypothyroidism, the reduction in the size of goiters, the body weight, the excretion of nitrogen, the viscosity and refractive index of the blood, the reaction of the nerves to stimulation, the toxicity of acetonitril in the mouse, the basal metabolic rate, the physiological effects of lack of oxygen, carbohydrate metabolism, the condition of the skin, the plumage of birds, the activity of certain cells in the animal organism and of unicellular organisms, the metamorphosis of amphibian larvæ and the silkworm, and the growth and changes in the organs of young rats.

Few of these effects were known in the earlier stages of thyroid investigation and the only criteria which were used by Baumann and by those contemporary with him were the changes in the size of goiters in man, the relief of hypothyroid symptoms, the increase in nitrogen elimination and the loss in body weight. As knowledge of the thyroid gland increased it became more and more desirable to have available a method of assay which would give an accurate index of the pharmaceutical value of various samples of desiccated thyroid.

Hunt [242] has found marked variations in the therapeutic value of thyroid preparations. The doses of desiccated thyroid mentioned in clinical reports have little significance. Not all samples are analyzed for iodine and even those that are analyzed may not be comparable in their physiological activity. Such a situation would not be tolerated with other drugs. The explanation is found in the difficulty attending the determination of the therapeutic value of any sample of desiccated thyroid. Of all the physiological reactions which have been investigated it is obvious that only those which are specifically influenced by thyroid material are available as a criterion of the activity of this gland.

It has been shown that the tadpole test is invalid when it is neces-

sary to prove the presence of thyroid material since a number of iodine compounds, not thyroxine, and not derived from thyroid substance will bring about the metamorphosis of the tadpole. The influence of the thyroid on the excretion of nitrogen is not specific since the administration of large amounts of iodide or of small amounts of certain amines and of hydrocyanic acid will result in increased elimination of nitrogen. Changes in physiological reactions such as the production of anoxemia and severe hemorrhage will also cause an increase in nitrogen output. Administration of known samples of thyroid material should produce variations in the nitrogen output in accordance with the activity of the material, and this method has been used in the past to test the relative activity of different samples of desiccated thyroid. A negative result is good evidence of the absence of thyroid material.

To all except three of the other physiological effects of thyroid material valid objections can be raised which prevent their use for the determination of the value of any particular thyroid preparation. The three possible methods are: the influence of the thyroid on the basal metabolic rate of patients with hypothyroidism or on thyroidectomized animals; the effect on the growth of small animals; and the effect on the resistance of the white mouse to acetonitril. Probably the most desirable method would be the effect on the adult human being which is in a completely hypothyroid state. For small quantities the influence of thyroxine in such an individual is in proportion to the amount administered and, since 1 mg. brings about a rise in basal metabolism which is equal to between 2 and 3 per cent of the normal metabolic rate, the method furnishes the most accurate possible index of the relative activity of various samples of desiccated thyroid. Whether or not a similar method can be applied to an experimental animal after thyroidectomy has not been conclusively shown. Thyroidectomized swine (Caylor and Schlotthauer) appear to offer the best chance of success. It is obvious that so long as this method requires the use of the hypothyroid human being it can never be used extensively.

The second possible method for standardization is the effect of thyroid material on small animals. Rainer has suggested the lethal dose of thyroid material, when fed daily in 0.2 gram amounts to a guinea pig weighing between 200 and 250 grams, as an index of the physiological activity of the preparation. Cameron and Carmichael [95] have carried out an extensive investigation concerning the relative activity of various thyroid preparations. They utilized the effect on the growth rate and production of organ hypertrophy in the white rat. It is interesting to

note that by this method results were secured which compared favorably with the results obtained by the use of Hunt's acetonitril method; and, although larger amounts of material are required, the influence on the growth of the white rat undoubtedly gives an accurate index of the physiological activity of the material.

Because of the unsatisfactory condition existing at present in regard to the standardization of thyroid material, Straub and Hunt were requested by the Health Committee of the League of Nations to consider the various methods which had been proposed for the biological assay of thyroid, with special reference to the acetonitril method. Hunt [241] has pointed out that such a test is highly desirable. The analysis of thyroid material for its iodine content is not satisfactory since potassium iodide or various other iodine compounds can be mixed with the desiccated thyroid. If desiccated animal proteins were mixed with stable organic iodine compounds it would be extremely difficult to prove that the iodine was not present in the unique form of combination in which it occurs in the thyroid. Analysis of the gland for thyroxine does not give any information concerning the physiological activity of the gland since it has been shown that not more than 15 per cent of the total iodine can be isolated in the form of thyroxine and in the majority of fresh thyroid glands the amount of the total iodine which can be separated in the form of thyroxine is not greater than from 5 to 10 per cent. Thyroxine cannot be separated from many samples of desiccated thyroid. Determination of the percentage of the total iodine which is insoluble in acid after alkaline hydrolysis is unsatisfactory since many iodine compounds are insoluble in acid.

Hunt and other investigators have shown that the action of thyroid material on the acetonitril reaction is specific to a remarkable degree. In contrast to the effect of many iodine compounds on the metamorphosis of the tadpole there is no other iodine compound which approaches thyroxine in its effect on the resistance of the white mouse to acetonitril. Hunt has shown that even di-iodotyrosine will not protect the white mouse against acetonitril if used in amounts comparable to the weight of thyroid necessary to protect this animal against two or three times the lethal dose.

Straub and Hunt, after a careful review of all the available methods, have concluded that the acetonitril is by far the most satisfactory test of thyroid activity. The advantages of this test and the essential requirements of any method of standardization are the extremely small

amount of material necessary and the high specificity of the substance to be tested.

It is unfortunate that the chemical reactions involved in this test are so obscure that they cannot be explained in terms of changes in basal metabolic rate or any other of the known effects of thyroid medication. The fact remains, however, that under proper conditions the white mouse varies in its susceptibility to acetonitril according to the amount of thyroxine which is administered, and for the present it undoubtedly gives the most accurate information which can be obtained concerning the physiological activity of various thyroid preparations.

Hunt [241] has suggested that the acetonitril test be based primarily on the iodine content and that samples of desiccated thyroid should be administered to mice in such amounts that the same weight of iodine will be present in each dose, regardless of the concentration of the iodine in the desiccated thyroid. The sample in a single dose should be given by stomach tube and the acetonitril should be given intravenously. He further suggested that simple chemical tests be included for the detection of non-thyroid iodine. The acetonitril test in combination with chemical tests should provide the means for satisfactory standardization of desiccated thyroid on the market. Hunt [242] has shown that the physiological activity of desiccated thyroid, as determined by the acetonitril test and by clinical observations, is closely parallel to the iodine content. Very few of the thyroid preparations on the market, however, were found to comply with the United States Pharmacopeia standard for iodine content and they were shown to vary greatly in their physiological and therapeutic activity.

Cameron [89] has also emphasized the great need of a method for the biological assay of thyroid preparations and has carried out a comparison of various thyroid preparations, with the rat-feeding test as a criterion. He has also compared the iodine content of several preparations of desiccated thyroid purchased in the market. The iodine content was found to vary within wide limits. It is necessary not only to insist on a minimal iodine value, but also on a maximal iodine value. Cameron believed that 0.4 per cent iodine in desiccated thyroid was too high and recommended standardization of all desiccated thyroid within the limits of 0.19 to 0.21 per cent.

Haffner and Komiyama have compared the various methods of standardizing thyroid preparations. They found that the amount of thyroid material which produced 100 per cent increase in the protection against acetonitril gave the most accurate index as the curve of response

was more linear at this point. They believed the method used in this way is the most practical one for standardization of thyroid preparations.

If the use of desiccated thyroid is continued, it is evident that it should be carefully standardized by biological methods. There seems, however, to be no apparent reason why thyroxine cannot be used to supplant desiccated thyroid completely. If this is so, a biological assay of the material is unnecessary: the chemical properties of thyroxine permit its preparation in pure form. A condition has not been found in which desiccated thyroid is essential, and it has been conclusively shown that the administration of thyroxine will bring about all the physiological changes induced by desiccated thyroid.

SUMMARY

The standardization of thyroid material is difficult. No single chemical test is adequate: a biological assay is necessary. There are three possible reactions which can be used. These are: the influence of thyroid material on the basal metabolic rate; on the growth and organ hypertrophy of young rats; and on the toxicity of acetonitril. The acetonitril test combined with chemical tests for added iodine compounds is the most satisfactory.

Chapter 16

The Physiological Activity of Various Preparations from the Thyroid Gland

The relative activity of preparations from the thyroid gland has been a problem of the greatest interest ever since Baumann first fractioned the constituents of the gland and demonstrated the activity of iodothyrin. Due to the faulty criteria which were at first used the conclusions drawn concerning the activity of preparations from the thyroid gland were incorrect. For many years the relation between the thyroid and parathyroid glands was not recognized, and variations in the condition of the experimental animal were undoubtedly the cause of some of the discrepancies encountered. This was particularly true when thyroidectomy had been carried out with varying degrees of trauma to the parathyroid glands. Variations in the chemical composition of some of the preparations from the thyroid gland explained some of the other discrepancies which were found. Baumann [33, 35] believed that iodothyrin could not be prepared from the thyroid gland of hogs and explained in this way the failure of Gottlieb to demonstrate the physiological activity of iodothyrin.

Fränkel [161, 162] was apparently misled in the preparation of thyreo-antitoxin by variations in the constituents of the protein-free extract of the thyroid after precipitation with acetic acid. It is quite possible that some of the acetic acid filtrates contained sufficient active thyroxine to bring about physiological effects; other preparations, freer from iodine, would be physiologically inert.

Thyreo-antitoxin: Fränkel's thyreo-antitoxin is an almost protein-free solution obtained by precipitation with acetic acid and lead acetate followed by removal of the lead with hydrogen sulfide. Magnus-Levy [329, 332] concluded that the thyreo-antitoxin which he tested had no effect on basal metabolism. This preparation of the thyroid gland was at first tested by Fränkel through its effect on the body weight and the production of hyperthyroid symptoms. It has been used but little and is

no longer available in the market: its method of preparation excludes any appreciable concentration of the active constituent of the thyroid.

Thyreoglandol: Thyreoglandol is the name of a proprietary preparation of the thyroid gland made by Hoffmann and LaRoche. It is described as an almost protein-free iodine-poor solution which contains the products obtained by perfusion of the gland. It has been used principally by Asher and his coworkers.[82] It is obvious that the physiological activity of a preparation made by perfusion of the gland will vary within wide limits, depending on the iodine content and the condition of the gland. Perfusion of some glands undoubtedly would extract traces of active thyroxine and the solution would possess physiological activity, whereas perfusion of glands which contained no, or practically no iodine would not give a solution with comparable physiological activity. One may feel secure in the conclusion that a preparation of thyreoglandol which did not contain iodine would not influence the metamorphosis of the tadpole, the basal metabolic rate, the rat-feeding test, or the acetonitril test.

Iodothyreoglobulin: The only substance, derived from the thyroid by simple extraction, which has uniformly produced physiological effects, is iodothyreoglobulin. This iodine-containing protein has been found active by all the tests which have been shown to be of value. Recently both Cameron and Carmichael [95] by the rat test, and Hunt [242] by the acetonitril test, have shown that iodothyreoglobulin possesses approximately the same physiological activity as desiccated thyroid.

Although Oswald [380] showed that tryptic digestion of iodothyreoglobulin completely destroyed its activity, Cameron and Carmichael [95] have shown that, provided the action of the enzyme is limited to a short time, both the soluble and insoluble portions of tryptic and peptic hydrolysis retain physiological activity of the same order as the original iodothyreoglobulin.

Iodothyrin: Iodothyrin does not possess physiological activity which is uniformly equivalent to that of desiccated thyroid when compared on the basis of its iodine content. This is true irrespective of the criteria used. Pick and Pineles found that the sample of iodothyrin which they used did not manifest physiological activity which was at all comparable to that of desiccated thyroid. Many samples of iodothyrin were feebly active when investigated by means of the acetonitril test (Hunt [239]). Jensen found that four different preparations of iodothyrin produced unequal effects on the axolotl when the samples were administered in amounts proportional to their iodine content. Magnus-Levy [332] showed

that iodothyrin affected basal metabolism in a manner qualitatively but not quantitatively the same as desiccated thyroid. Koch [282] found that with the acetonitril test as a criterion a sample of iodothyrin which he prepared possessed about three-fourths the activity of desiccated thyroid when compared on the iodine content.

The amount of the total iodine in the gland which can be obtained in the iodothyrin fraction is not greater than 25 per cent. Since in no case is iodothyrin more active than desiccated thyroid when compared on the basis of its iodine content, the iodine-containing compounds not separated as iodothyrin cannot all be physiologically inert. It therefore follows that during the hydrolysis of the thyroid material with 10 per cent acid the physiologically active iodine compounds in the gland must be in part decomposed. This occurs in at least two ways: in one acid-soluble products are formed which are physiologically inert, and in the other acid-insoluble and alcohol-insoluble products are formed which are almost, if not quite, physiologically inert. In addition to these two groups of decomposition products there may be several different iodine-containing compounds in iodothyrin. This fraction from the hydrolysis of the thyroid proteins can be separated because it is insoluble in aqueous acid and is soluble in alcohol. These two properties are the only ones which characterize this preparation and they are not sufficient to bring about a chemical separation of physiologically active compounds from other iodine-containing substances which possess no activity. It is significant that the maximal iodine content of iodothyrin which has so far been prepared is about 14 per cent. Since thyroxine contains 65 per cent of iodine, iodothyrin cannot contain more than 25 per cent of thyroxine. The probable structure of iodothyrin is that of a polypeptide, one constituent of which is closely related to thyroxine. I have prepared iodothyrin from desiccated thyroid which possessed marked physiological activity but from which crystalline thyroxine could not be separated after hydrolysis with sodium or barium hydroxide.

Products soluble and insoluble in acid after alkaline hydrolysis: Roos,[432] in 1896, showed that, when the thyroid gland is boiled with acid, the products of hydrolysis possessed greater physiological activity than when the same material was boiled with alkali. According to the criterion which was at that time used (the reduction in the size of goiter in man) the products of alkaline hydrolysis appeared to be physiologically inert. Other tests concerning the action of alkali on the thyroid were not carried out until I investigated the acid-insoluble fraction of

alkaline hydrolysis. These products were shown to manifest each and every physiological effect produced by desiccated thyroid.

Careful investigation of the acid-soluble compounds after hydrolysis with alkali has shown that this fraction does not produce any effect similar to thyroid material. This statement is true irrespective of the criterion which is employed. From the chemical investigation it seemed probable that these iodine-containing compounds are intermediate substances in the synthesis of thyroxine. It is possible that di-iodotyrosine is contained in this fraction but the physiological activity of this substance is not greater than one-thousandth of that of thyroxine. It is remarkable that thyroxine can be so completely separated from the other iodine-containing compounds in the gland merely by its solubility in aqueous acid. That this is true, however, has been abundantly demonstrated.

Thyroxine: The qualitative and quantitative relation between the physiological activity of pure crystalline thyroxine and that of desiccated thyroid is of the greatest significance. Thyroxine has been subjected to practically every possible criterion. In every respect it has been found to be the full equivalent of desiccated thyroid. Not only is every qualitative reaction produced but even quantitatively the relations are well within the limits to be anticipated. It must be clearly recognized that thyroxine does not bring about a physiological response until many hours after it has been injected: some deep-seated reactions are essential before thyroxine can be incorporated in the protoplasm and assume the correct physical and chemical relationships which are necessary for the production of its physiological activity. It is remarkable that this can be accomplished with such a high percentage of the total amount of thyroxine administered. With small doses there is practically no thyroxine eliminated in the urine and the amount lost through the bile must be a very small percentage of the total.

Demonstration that the entire physiological activity of desiccated thyroid is produced by the single molecule, thyroxine, affords but a partial explanation of the physiological activity of iodine compounds in the thyroid gland. Since not more than about 25 per cent of the total iodine in the thyroid gland can be separated in a form which is physiologically active after alkaline hydrolysis, it is incontrovertible that a large part of the physiologically active iodine-containing compounds are destroyed with sodium hydroxide. The explanation which will be given at this time is that the total activity of the thyroid gland is brought about by thyroxine which exists in the gland in the form of this compound as isolated, and in addition in a slightly altered form which will be

described as "active" thyroxine. Not only is it highly probable that before thyroxine can function at all it must assume the active form but it also appears certain that the physiological activity of the thyroid is in fact due to active thyroxine only. The physiologically active form of thyroxine cannot differ from thyroxine merely through the linkage of thyroxine in the protein by means of the carboxyl or the amine group. There must be a more radical alteration in the structure of the molecule. Sufficient evidence for this is the fact that thyroxine is stable to sodium or barium hydroxide and yet hydrolysis with these alkalies of some samples of desiccated thyroid which possess marked activity destroys the iodine-containing compounds: no thyroxine can be isolated.

Hunt [242] has asked the question whether there is physiologically inactive iodine in the gland and has answered it in the negative; this conclusion was reached after it had been shown that desiccated thyroid possessed about one and one-half times the physiological activity of pure, crystalline thyroxine (Hunt [239]).

Cameron and Carmichael [94, 95] have also demonstrated that crystalline thyroxine possessed physiological activity which was somewhat less than that of desiccated thyroid when compared on the basis of the iodine content. Hunt's suggestion that the iodine-containing compound in the thyroid exists originally in a more highly iodized state, and that some of the iodine is broken off during the separation of the compound is not satisfactory. An alternative explanation, which as yet lacks quantitative proof, will be suggested with the full realization that it furnishes merely a sketch which may have to be abandoned. Within a comparatively short time it may be possible to reject it completely or to amplify the sketch with fuller detail. There is need for a working hypothesis and without further apology the following tentative suggestion is made.

The physiologically active form in which thyroxine functions may be identical with thyroxine except for the presence of a second hydroxyl group, probably in the outer benzene ring. This compound would bear to thyroxine the same relationship which exists between di-oxyphenylalanine and tyrosine. It is known that di-oxyphenylalanine is destroyed by alkalies; however, tyrosine can be boiled with alkali and recovered. If thyroxine can exist as a dihydroxy derivative, that portion which is present in the dihydroxy form would be converted into products which could not be recovered after alkaline hydrolysis. On the other hand, this substance would be stable to acid hydrolysis, although it is probable that it would be more difficult to separate in crystalline form because of its greater solubility. If this hypothesis is correct, then it is obvious that

the dihydroxy derivative could exist in two forms, which would bear to each other the same relationships which exist between resorcinol and its corresponding quinone; the substance could exist in either oxidized or reduced form, and here again stability to hydrolysis would be different.

Finally, a third possibility exists: that two atoms of iodine are attached to the inner benzene ring of thyroxine and none is attached to the outer ring. However, the outer ring may exist as a dihydroxy derivative which would also have oxidized and reduced forms. This series of four compounds, all of which contain iodine, would all be physiologically active when combined with the protein molecule in the thyroid secretion. Acid treatment would destroy and alter them to an unknown degree largely depending on the conditions present, but from experimental data we know that under most favorable conditions at least 75 per cent of these iodine compounds are rendered physiologically inert. Sodium hydroxide would destroy even more and leave thyroxine as the only physiologically active product which could be recovered.

Cameron and Carmichael [95] have shown that sodium hydroxide destroyed at least two-thirds of the activity of iodothyreoglobulin. This applies to the sample of iodothyreoglobulin which they used. The results of an extensive investigation which I carried out concerning variations in the thyroxine content of various samples of desiccated thyroid leave little doubt that alkali would destroy far more of the physiological activity of other samples of iodothyreoglobulin; in fact, alkali may destroy practically 100 per cent of the physiological activity of some samples of desiccated thyroid. The assumption, therefore, that the iodine in the acid-insoluble products after alkaline hydrolysis is a measure of the thyroxine content of the gland is incorrect and the amount of thyroxine which can be isolated from the gland has no significant relation to the physiological activity which the thyroid material may possess before hydrolysis with alkali.

There is at present a wide discrepancy between the suggestion of Hunt that the thyroid does not contain any iodine compound which is physiologically inert, and the results which have been obtained by alkaline and acid hydrolysis of the thyroid. From the chemical nature of thyroxine it is impossible to assume that either dilute alkali or acid can bring about a destruction of this compound. A most characteristic property of thyroxine is its insolubility in aqueous acid. When thyroid material is subjected to either acid or alkaline hydrolysis more than half of the total iodine is found soluble in acid. This fraction does not possess physiological activity of any kind. It is difficult to believe that

all of the iodine compounds which are soluble in acid are formed by decomposition of iodine derivatives of oxyphenyltyrosine.

In addition to the chemical differentiation of the iodine-containing compounds in the thyroid and the very suggestive evidence adduced by Ingvaldsen and Cameron,[248] that di-iodotyrosine may be present, biological evidence concerning the chemical nature of these compounds is not lacking. Kendall [270] and Krayer have shown that thyroxine was stable when injected into the animal organism. Veil and Sturm have recently shown that iodine was not excreted in the urine after the injection of thyroxine. On the other hand, Blum and Grützner have shown that iodothyreoglobulin was decomposed not only in the living organism but also (*in vitro*) by the liver. Oswald [381] found that in the rabbit di-iodotyrosine was in part broken down into inorganic iodide. Veil and Sturm showed that after the administration of iodothyreoglobulin a large percentage of the iodine was excreted in the urine. Of the iodine excreted, 33 per cent was in the inorganic form. Krayer found iodine in the urine after the administration of di-iodotyrosine and then made the significant observation that the distribution of iodine following the administration of iodothyreoglobulin was similar to that following the injection of di-iodotyrosine. These biological results indicate that the animal organism hydrolyzes the proteins of the thyroid as do mineral acids or sodium hydroxide. The data offer further indication that all of the iodine in the gland is not in the same form of combination and the results are incontrovertible evidence that all the iodine in the gland is not in the form of thyroxine. The observations are in keeping with the chemical differentiation which I have reported: between 40 and 50 per cent of the total iodine in the thyroid gland is present in a physiologically inert form.

Opposed to these results concerning the presence of physiologically inactive iodine compounds are the observations of Hunt,[240] that the activity of desiccated thyroid is greater than that of thyroxine when compared on the basis of their iodine content. This observation could be explained provided the effect of "active" thyroxine as it occurs in the thyroid gland was but three or four times greater than the effect of pure, crystalline thyroxine. The nature of the physiological response to thyroxine is such that there can be little doubt that the molecule must be first incorporated within the functioning protoplasm. If this step were incompletely carried out, then the physiological activity of the crystalline material would be quantitatively inferior to that of "active" thyroxine which existed in the necessary physical and chemical status. Further-

more, thyroxine as injected is in the racemic form and it is possible that the optically active form could easily possess two or three times the activity of the racemic mixture. *l*-Epinephrine has been shown to possess fifteen times the activity of *d*-epinephrine.

That the functioning form of thyroxine may possess more activity than the pure, crystalline substance is suggested by the extraordinary gap which exists between thyroxine and all other known iodine compounds in respect to the effect on the toxicity of acetonitril. Even di-iodotyrosine has been shown to possess physiological activity which is about one-thousandth that of thyroxine, and yet di-iodotyrosine is an integral part of the thyroxine molecule. Since thyroxine possesses such unique power, when measured with the Hunt test, it is not improbable that slight modifications in the molecule could increase its activity to a value three or four times that observed for the crystalline compound. The derivatives investigated by Gaddum which closely approach the configuration of thyroxine were shown to possess activity which varied from no effect to about one-fortieth that of thyroxine.

These results indicate that the conclusion that the thyroid gland does not contain any physiologically inactive iodine cannot be made solely from experiments of the type which have been carried out up to the present. Evidence has not been submitted which disproves the statement that possibly half of the total iodine contained in the thyroid gland is present in a form which is physiologically inert.

Although thyroxine probably does not function in the form in which it is isolated, whatever alteration is required is brought about by cellular activity other than that of the thyroid gland itself. Pure, crystalline thyroxine administered in a case of high-grade myxedema or to an animal after thyroidectomy becomes physiologically active.

Thyroxine undoubtedly represents an intermediate stage in the elaboration of "active" thyroxine. Since desiccated thyroid may not contain crystalline thyroxine, and since other samples of thyroid material may contain as much as 15 per cent of the total iodine in the form of thyroxine, it seems probable that this compound may be altered chemically after the thyroid gland has been removed from the animal but the chemical change is not to a degree sufficient to destroy its physiological activity. I have shown that significant changes in the chemical properties of the various iodine compounds are brought about by allowing fresh thyroid material to stand.

The knowledge that thyroxine is derived from di-iodotyrosine enables the chemist to understand all of the steps in the synthesis of this essen-

tial substance from an amino acid and iodine which occur in the food. For the determination of the chemical nature of the iodine-containing compound of the thyroid, it is indeed fortunate that it exists at least in one stage which is so stable to alkali that it can be separated from the protein by hydrolysis. This fact has permitted an attack on the problem of the "active" form.

In this respect it may be compared to radium which exists in one stage as a gas. While in this form it may be separated from solution and concentrated within a small volume which possesses quantitatively practically the entire equivalent of the available radio-activity produced.

SUMMARY

The physiological activity of the following preparations from the thyroid gland have been studied: thyreo-antitoxin, thyreoglandol, iodothyreoglobulin, iodothyrin, acid-soluble and acid-insoluble fractions after alkaline hydrolysis, thyroxine. Thyreo-antitoxin and thyreoglandol may not produce the characteristic influence of the thyroid on the basal metabolism. The methods of preparation do not insure a significant concentration of the iodine compounds in the thyroid. Iodothyreoglobulin possesses physiological activity qualitatively and quantitatively the same as desiccated thyroid when compared on the basis of the iodine content. Iodothyrin produces qualitatively the same results but quantitatively it is much weaker when compared on the basis of its iodine content with desiccated thyroid. The fraction of thyroid material which is insoluble in acid, after alkaline hydrolysis, possesses qualitatively all of the activity of desiccated thyroid, quantitatively it is much inferior.

Thyroxine produces qualitatively every physiological effect of desiccated thyroid; quantitatively it is somewhat less active. Certain samples of desiccated thyroid with marked physiological activity do not contain any thyroxine which can be separated in crystalline form. This suggests that the total activity of the thyroid gland is brought about by thyroxine which exists in the gland in the form of this compound as isolated, and in addition in a slightly altered form, "active" thyroxine. Before thyroxine can function it must assume an active form. Besides thyroxine and active thyroxine there are probably other iodine-containing compounds which are physiologically inert. This conclusion is based on the distribution of iodine in the fractions obtained by alkaline and acid hydrolysis and the distribution of iodine excreted in the urine after administration of thyroid. Active thyroxine may contain a second hydroxyl group in the outer benzene ring.

Chapter 17

The Relation Between the Histological Appearance and the Physiological Activity of the Thyroid Gland

There can be little doubt from the many investigations which have been carried out concerning the relation between the diet and the activity of the thyroid gland that stimulation or atrophy of this organ is directly dependent on the amount and the composition of the diet. Bensley[39] has shown that spontaneous hyperplasia of the thyroid gland of the opossum can be produced and controlled by diet alone. Burget found that if adult rats were kept under hygienic conditions and fed a high-protein diet hyperplasia of the thyroid gland developed. If they were kept under unhygienic conditions hyperplasia of the thyroid gland developed if a standard diet of bread and milk was given. Unhygienic conditions and a high-protein diet brought about a higher degree of hyperplasia in the adult rat than either factor taken alone. If young, growing rats were kept under hygienic conditions hyperplasia of the thyroid did not develop when a high-protein diet which would induce this condition in adult rats was given. Feces either from goiter patients or from goitrous dogs, when given to the cat by stomach failed to induce any changes in the thyroid gland.

Tsuji has also demonstrated significant changes in the histological appearance of the thyroid which were produced by a deficient diet. These changes were attributed to the diminished function of the gland which in turn induced a profound change in many of the organs in the animal. The changes in the other organs were found to be the opposite to the effect produced by the administration of thyroid material. He found hypertrophy of thyroids of rats fed on a large amount of fresh egg-yolk or fresh milk. The increased activity caused hypertrophy of various organs of the body. The histological features of these organs were similar to that produced by thyroid feeding; on the other hand, the same organs of a thyroidectomized animal showed atrophy or degeneration, analogous to the changes found in hypothyroidism produced by

deficient diet. From these data, Tsuji concluded that fresh egg-yolk or fresh milk contains a substance or substances which stimulate the thyroid gland.

McCarrison [324] has determined the effect of various amino acids on the condition of the thyroid gland and metamorphosis of the tadpole. Tryptophane appeared to increase the size and colloid content of the thyroid gland. The thyroids of the tadpoles fed histidine contained the least colloid. He [325] has recently published results of an investigation concerning the production of goiter in rats. He found that goiter was caused by a diet which contained more than 60 per cent of white flour or of vitamine-poor carbohydrate, 20 per cent or less of protein with fats and inorganic salts, including iodine, in adequate amounts, but no green vegetables or fruit. Goiters produced with this diet were not relieved by the administration of iodine. If the same stock of rats were given a proper diet goiter did not develop.

Another factor which was suggested several years ago and has been extensively investigated is the influence of bacteria. McCarrison [322] has assembled the evidence concerning the relation between bacteria and infection, and the incidence of goiter in India. In a more recent article, he [323] expressed the belief that goiter was due to the relative requirements and supply of iodine. Under unhygienic conditions such as a polluted water supply more iodine is required in order to preserve a normal histological structure and function of the gland. Plummer [400] had previously expressed the same conclusion: "the relation of the bacterial flora to the available iodine would well explain the geographic distribution of endemic goiter."

Clinicians have suggested that the development of goiter and the severity of the symptoms of hyperthyroidism are dependent on infection in other parts of the body, such as the teeth and tonsils. It has been found that cases of goiter are sometimes relieved by removal of the teeth and tonsils when these are infected.

THE RELATION BETWEEN IODINE AND COLLOID

The use of iodine in the treatment of goiter dates back to a time before the discovery of iodine as an element. The distribution of iodine in its relation to goiter has been recently reviewed by McClendon. Kocher [285] first suggested the desirability of investigating the relation between administration of iodine and thyroid activity, and after iodine was discovered as a constituent of the gland a more logical basis for the treatment of the gland with iodine preparations became apparent.

Bruns, as early as 1896, treated various forms of goiter with thyroid material. He showed that the administration of desiccated thyroid apparently relieved the load under which the gland was laboring. He suggested that the effect of the administration of thyroid was to bring about the sloughing and atrophy of a part of the functioning acini.

Fordyce [158] showed that the administration of thyroid material to rats brought about changes in the thyroid gland which indicated diminished activity. Bensley found that the administration of iodides to animals with thyroids in a hyperplastic state caused the storage of intrafollicular colloid. Baumann,[34] Oswald,[377] Marine and Williams, Carlson and Woelfel,[100] and many others have investigated the iodine content of goiters and endeavored to relate the physiological activity with the histological appearance of the gland and its colloid content.

Aeschbacher suggested that the colloid which is found within the acini as a thick, deeply-staining material is the principal carrier of the iodine, and that variations in the staining properties of the colloid indicate varying iodine content. He found that acute infections brought about, in a large number of cases, a decided diminution in the iodine content. This he felt was due partly to the disappearance of colloid and partly to a decrease in the iodine content of the colloid.

Marine and Lenhart [388] studied a large series of thyroid glands removed from different animals and compared their iodine content and the structure of the thyroid. They also studied [389] the effect of iodine on goiters which were in an active state and showed that the administration of iodine hastened the change from the hyperplastic condition in which there was but little colloid and brought about deposition of colloid material within the gland. They concluded that iodine could bring about this change in all types of goiter in which the gland existed under various degrees of stimulation.

Marine and Lenhart maintain that the minimal amount of iodine necessary for the normal histological structure of the thyroid gland averages about 1 mg. for each gram of dried material. It seems probable that this statement must be modified to the extent that the diet and the temperature have marked effects on the activity and structure of the thyroid. When sheep are given a diet, as in the mountains of Montana, during the summer months, far less than 1 mg. of iodine for each gram of tissue may be required to maintain a normal functioning gland, but when these sheep, during the winter months are given a concentrated, fattening diet, which is free from fresh, green material, larger amounts

of iodine may be required to preserve the normal histological appearance of the gland structure.

Sugata suggests that endogenous protein metabolism is fundamentally responsible for the distribution of iodine in the body, and that there may be a type of iodine equilibrium throughout the body: the actual distribution may be dependent in some way on the metabolism.

Marine and Rogoff [340] showed that twenty hours after the injection of 50 mg. of iodine definite histological changes could be found. The greater the degree of stimulation indicated by histological appearance the more readily were the changes detected. The rate of metamorphosis of the tadpole was definitely increased eight hours after the injection of potassium iodide. Twenty hours after the administration the effect on the gland was marked. This indicated that morphological changes were closely related to the presence of the iodine-containing hormone. The thyroid took up iodine almost instantaneously, while the elaboration of the hormone was slow.

Claude and Blanchetière [104] expressed the opinion that iodine exists in a transitory state before it is transformed into iodothyreoglobulin. They state that the iodine content and the amount of colloid are not parallel, and that all degrees of disproportion exist. There may be much colloid and no iodine, or much iodine and no demonstrable colloid. On the other hand, Thomas and Delhougne have reported a parallelism between the iodine content and the colloid in most cases.

Wegelin and Abelin tested the biological activity of the glands of man by their influence on the tadpole. They found that neither the amount nor composition of the colloid influenced the activity of the gland. Abelin [6] has recently carried out a study in which the biological activity of goiters was studied through the tadpole test. The iodine content and histological condition of the gland were also determined. He found that calf-fetus glands contained iodine and were biologically active. Human-fetus glands usually contained iodine, but did not hasten metamorphosis of the tadpole. In two cases where the mother had been given potassium iodide the fetal gland was normal as to histologic structure, colloid content and biological activity. These glands also contained a normal amount of iodine. Diffuse goiter usually contained colloid, had a high iodine content, and produced a marked effect on the metamorphosis of the tadpole. In adenomas of the thyroid the iodine content varied but it was usually lower than in *"struma diffusa."* The biological activity of this type of goiter was independent of the colloid and of the iodine content. There was no parallelism between biological activity

(tadpole test) and the amount of colloid, between the amount of colloid and iodine, or between the iodine content and the biological activity. He found that in all cases of exophthalmic goiter with marked clinical symptoms there was relatively little iodine and no biological activity as indicated by the metamorphosis of the tadpole. Proliferating (Langhan's) goiter and the metastatic nodules of thyroid adenoma in bone contained iodine which may be biologically active. The metastatic nodules contained more iodine than the proliferating goiter. Iodine was present in some glands which did not contain colloid material when examined histologically. He therefore suggested that the active constituent is not completely elaborated in the acini and held in the colloid: the iodine must also be present in the epithelial cells.

An interesting and important investigation concerning the distribution of iodine in the thyroid gland was initiated by Tatum. By cutting the thyroid gland in thin sections it was found possible to dissolve the colloid content of the acini in physiological salt solution and leave the cells of the thyroid suspended in the solution. After separation of the cells from the solution the iodine content of each fraction was determined. By the use of this fractionation he hoped to obtain definite information concerning the elaboration of the iodine compounds and to study the distribution of these compounds in various conditions of goiter. It was shown that the ratio of the percentage of the iodine in the cells to the percentage of the iodine in the desiccated gland was a fairly constant value.

Van Dyke [510, 512] continued the investigation and showed that feeding potassium iodide or tincture of iodine did not materially alter the ratio of the percentage of the iodine in the cells to the percentage of the iodine in the desiccated gland, after an interval of three days. The ratio was found to be very low after the intravenous injection of potassium iodide into dogs with hyperplastic glands when the glands were removed one and a half to sixty minutes after the injection. The ratio for the glands of uninjected dogs was found to vary between 0.115 and 0.367, and was usually about 0.15. The ratio was relatively constant despite variations in morphological characteristics and iodine content.

Williamson, Pearse and Cunnington have recently investigated the distribution and the physiological activity of iodine-containing compounds in thyroid glands with varying histological appearance. They assume that the thyroid gland may exist in a secretory phase and in a colloid phase. By the use of the method devised by Tatum they showed that in the secretory phase iodine may be present in a relatively high

concentration but is physiologically inactive; the iodine is contained within the cells. In the colloid phase the iodine is present in the alveoli and is biologically active when tested on the tadpole even though it is present in low concentration. The difference in the physiological activity they consider qualitative and not quantitative.

Wilson and Kendall [528] examined a large series of cases of goiter for their iodine content and histological appearance and compared the findings with the clinical condition of the patients. It was shown, in agreement with many other investigators, that the iodine content of exophthalmic goiter glands was by far the lowest, that the percentage of iodine in large colloid goiters was low, but that the total amount present might be high, and that there appeared to be no parallelism between the amount of the iodine in the thyroid gland and the severity of the hyperthyroidism as judged by clinical symptoms.

PREVENTION OF GOITER WITH IODINE

Besides the influence of iodine on the physiological activity of the gland and quite distinct from the problem of the various steps by which thyroxine is synthesized in the gland, one of the most important problems concerned with the investigation of the thyroid is the influence of iodine on various types of goiter. Knowledge of the chemistry and physiology of the thyroid gland is essential for the control of goiter. The results obtained through an investigation of the chemistry of the thyroid gland find practical application in the relief of goiter.

Some of the most important results in regard to the relief of endemic goiter have been obtained by Kimball and Marine [278] and Kimball, Rogoff and Marine.[279] They investigated the thyroid glands of the school children of Akron, Ohio, a city in a "goiter district." The results indicate that the danger of giving iodine to children in this country is negligible even if a small, colloid goiter is present. Striking results were obtained which clearly demonstrate the relief of this type of goiter through the use of iodine.

The success of Marine and Kimball and others in relieving endemic goiter was followed by the use of iodine in other countries and in other goiter districts in the United States. Silberschmidt has reported the results obtained in Switzerland. Kimball [277] recently reviewed this subject and pointed out the marked decrease in the incidence of goiter. He concluded with a statement that the goiter problem will be solved within another generation. While this optimism appears to be justified, there

are doubts in the minds of many clinicians concerning the advisability of treating all types of goiter with iodine.

Kocher [286] in 1910 pointed out that serious results apparently followed the administration of iodine in certain cases of goiter. Instead of bringing about a diminution in the size of the goiter, severe symptoms of hyperthyroidism were produced, and these symptoms persisted even after the administration of iodine was stopped.

Plummer [401] is not convinced that the cause of the symptoms of hyperthyroidism after the administration of iodine to patients with adenomatous goiter is directly related to the iodine. He says, "I am still of the opinion that the administration of iodine causes adenomatous thyroids to hyperfunction; that it does, however, is an inference, not an established fact. There is little, if any, evidence on which to assume that the administration of iodine will cause an increase in the degree of hyperthyroidism once hyperfunction has started; however, hypotheses urge caution."

Kimball [277] is convinced that the number of cases of goiter without symptoms in which a status of hyperthyroidism is induced by the administration of iodine is negligible. He regards the development of symptoms in the few cases which were observed as merely a coincidence. Certainly he can show a mass of statistics which sustain this view. Hartsock [205] expresses the opposite view, but his conclusions are based on only a few cases.

The results of the intensive study of this problem which is now being carried out will be awaited with much interest, not only by the clinician but also by the physiologist and biochemist. The problem deals with fundamental aspects of glandular activity which have a wide application.

Plummer [399, 401] has sharply differentiated the group of cases which is comprised of hyperfunctioning adenoma from those of exophthalmic goiter. The cause of the over-activity of an adenoma is not known, but Plummer [401] has pointed out that the average time required to produce this condition is measured in terms of years. Symptoms of hyperthyroidism usually appear in this group of cases from fourteen to seventeen years after the first appearance of the goiter.[399] After an adenoma has become over-active, the only relief which is known today is the surgical removal of the adenoma. It is not necessary to remove any part of the normal thyroid gland in this condition, and after the adenoma has been separated from the gland and the basal metabolic rate has

returned to normal, all objective and subjective signs of hyperthyroidism are relieved (Plummer [401]).

Investigations by clinicians and physiologists on the influence of iodine have established beyond controversy its beneficial effect in endemic goiter. There is another condition of the thyroid which is influenced by the administration of iodine, and the results obtained are of even greater consequence. Iodine given in cases of endemic or adolescent goiter may bring about regression in the size of the gland and improvement in the appearance and in some cases a change in the general condition of the patient. In strong contrast to these effects it has been

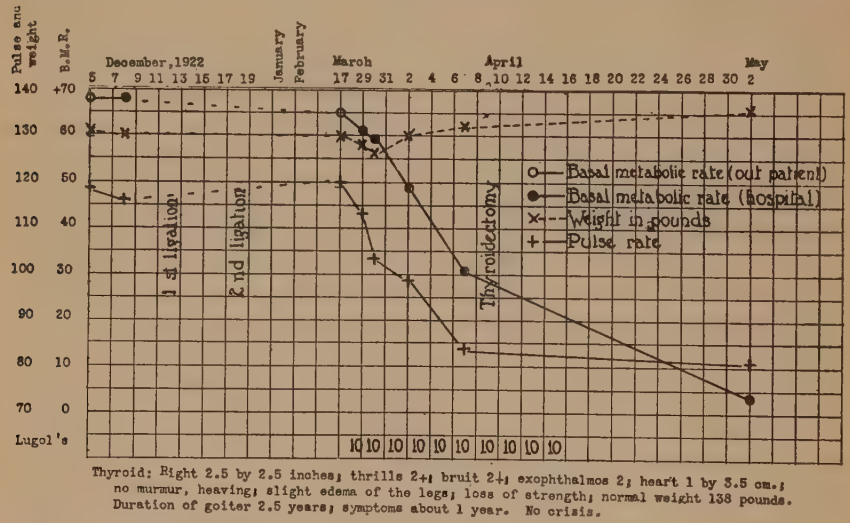


FIG. 36.—Influence of Lugol's solution on the basal metabolism of a patient with exophthalmic goiter. (Plummer and Boothby.)

found that the administration of iodine in cases of exophthalmic goiter may be a life-saving measure. The dangerous and in some cases critical condition of the patient may improve with dramatic rapidity.

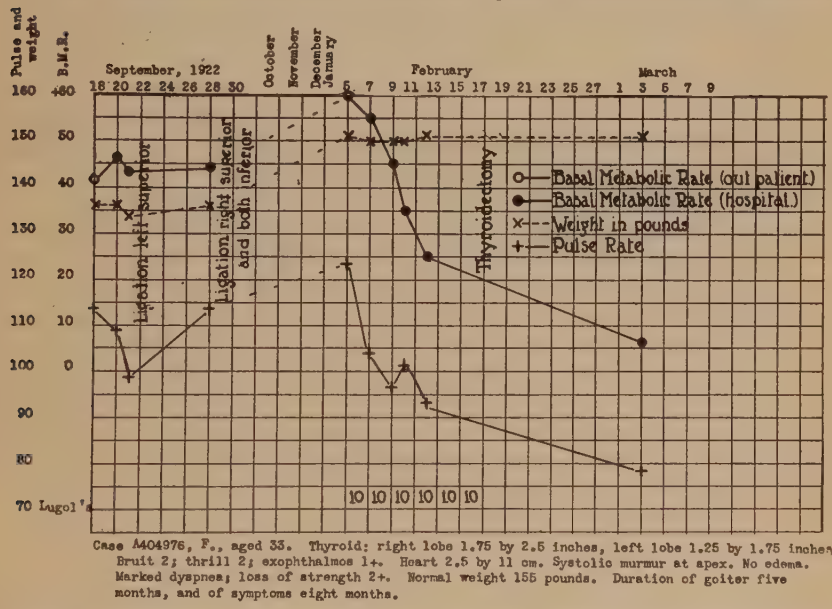
Neisser [367] and Loewy and Zondek, in 1922, reported a beneficial influence of iodine in cases of goiter. Independently and at about the same time, Plummer carried out an investigation on the influence of the administration of iodides and iodine in cases of exophthalmic goiter.

This work (Plummer and Boothby [404] and Boothby [63]) has shown that when Lugol's solution is given in exophthalmic goiter, there may be a marked drop in the basal metabolism with coincident relief of excessive nervousness and nausea, and if the patient is in the critical condition

which sometimes is seen in this disease, it is possible to bring about a remission of symptoms which permits surgical removal of the thyroid gland without undue risk. (Figs. 36, 37, 38.) The results of Plummer and Boothby have been confirmed by Starr and Means, [465] by Mason,[344] and by Cowell and Mellanby.

The relation of iodine to thyroid activity in clinical medicine has been reviewed by Bircher,[45] Graham,[180] Foster,[159] and recently by Breitner.[71]

The influence of iodine on the thyroid gland in this condition cannot



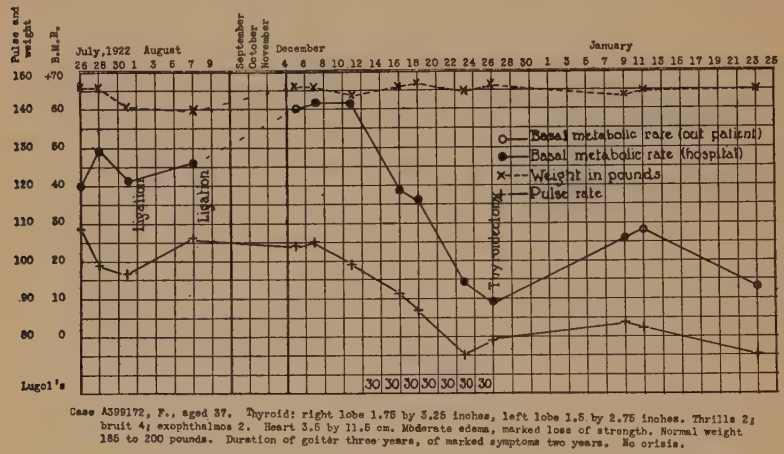
Case A404976, F., aged 33. Thyroid: right lobe 1.75 by 2.5 inches, left lobe 1.25 by 1.75 inches. Bruit 2; thrill 2; exophthalmos 1+. Heart 2.5 by 11 cm. Systolic murmur at apex. No edema. Marked dyspnea; loss of strength 2+. Normal weight 155 pounds. Duration of goiter five months, and of symptoms eight months.

FIG. 37.—Influence of Lugol's solution on the basal metabolism of a patient with exophthalmic goiter. (Plummer and Boothby.)

be expressed in terms of chemistry at present. An attractive hypothesis which appears adequately to explain the effect, however, assumes that iodine acts on the thyroid gland and not primarily in the tissues or other organs in the body.

Sturgis, Zubiran, Wells and Badger [475] have investigated the influence of iodine on the condition of hyperthyroidism produced by injection of thyroxine. They found that administration of iodine did not change the reaction to thyroxine in rabbits. Hildebrandt,[224] on the other hand, has reported an investigation on the basal metabolic rates of rats which has shown that these animals are affected by small amounts of potassium

iodide. He first showed that thyroxine manifested the specific effect of the thyroid gland and stated that its action was not due to the fact that iodine split off from the molecule, but to the configuration of the molecule. By the injection of 5 to 10 mg. of potassium iodide in normal rats Hildebrandt showed that small amounts of iodine caused a decrease in the basal metabolic rate which lasted for several days; larger doses, 30 to 50 mg., were toxic and caused death with convulsions. If the rat survived a larger dose the basal metabolic rate was increased. Rats which were fed desiccated thyroid were more sensitive to potassium iodide: 5 to 10 mg. of this material now produced a great increase in the basal metabolic rate, whereas the basal metabolic rate of rats which were not



Case A399172, F., aged 37. Thyroid: right lobe 1.75 by 3.25 inches, left lobe 1.5 by 2.75 inches. Thrills 2; bruit 4; exophthalmos 2. Heart 3.5 by 11.5 cm. Moderate edema, marked loss of strength. Normal weight 185 to 200 pounds. Duration of goiter three years, of marked symptoms two years. No crisis.

FIG. 38.—Influence of Lugol's solution on the basal metabolism of a patient with exophthalmic goiter. (Plummer and Boothby.)

fed thyroid material was decreased by this amount of potassium iodide.

Solé was unable to show any effect of potassium iodide when given in amount equal to the iodine contained in a dose of thyroid material which brought about a marked physiological response. Whether or not the rats used by Hildebrandt were in a susceptible condition is not apparent, or whether the rat reacts in a different manner than man and other animals is not now known, but the therapeutic use of iodide in man has not shown any of the marked effects reported by Hildebrandt. Moreover, the work of many other investigators on dogs and other animals has not confirmed the results of Hildebrandt.

There is valid evidence that the effect of iodine in exophthalmic goiter is not exclusively on the tissues, and that a large part of its action

is probably brought about through alterations of the chemical processes occurring in the thyroid gland and through an increase in the ability of the thyroid to retain its secretion.

One effect of iodine on the gland which can be readily shown is the retention of iodine by the gland. Rienhoff has shown that iodine produced definite and striking changes in the histological appearance of the glands in exophthalmic goiter which were directly associated with the clinical improvement and the decrease in basal metabolism. Giordano [174] and Sager [438] have shown definite changes in the colloid content and a decrease in the hypertrophy and hyperplasia in the thyroid after the administration of iodine.

Cattell [102] found that in from 90 to 95 per cent of the glands in exophthalmic goiter there was some degree of involution following the administration of iodides. The amount of iodine taken up appeared to increase to the saturation point, beyond which the gland was unable to store more iodine. Cattell suggested that iodine enabled the thyroid gland to approach a more normal histological appearance and function.

Weir has recently investigated a group of goiters which were removed after the administration of Lugol's solution. He found that not only was the iodine content increased but the total amount of acid-insoluble iodine-containing compounds, after hydrolysis with sodium hydroxide, was increased. The conclusion was drawn that this represented an increased percentage of the total iodine in the form of thyroxine. Whether this is correct or not, it is evident that the distribution of the various forms of the iodine-containing compounds was altered after the administration of Lugol's solution.

A question of great significance concerning the physiology of the thyroid gland and the rational basis for therapeutic treatment is the possibility of two types of hyperthyroidism: one, excessive discharge from the gland of the normal thyroid secretion (this would produce "pure" hyperthyroidism), and the other, the excessive discharge from the gland of an abnormal thyroid secretion (this would produce a condition which would differ in some respect from "pure" hyperthyroidism). This problem has been considered by clinicians and pathologists for many years and it has not yet been answered to the satisfaction of all. The names of Asher, Aschoff, Biedl, Bircher, Breitner, Ewald, Gley, Janney, Kocher, Marine, Means, Moebius, Plummer, Quervain, Schols, Warthin, Wilson and many others are associated with this question which cannot yet be answered in terms of chemistry. Before this aspect of thyroid

activity is discussed, certain related facts in clinical medicine will be presented.

SUMMARY

Goiter may be produced by improper diet or by unhygienic surroundings. Administration of iodine brings about histological changes and the deposition of colloid. Iodine may be present in the thyroid which does not contain colloid. Biologically inactive iodine may be present in the gland. There may be no parallelism between the biological activity and colloid, the amount of colloid and iodine, or between the iodine and the biological activity.

Iodine is of great value in the prevention and treatment of endemic goiter. In a small percentage of cases iodine may cause symptoms of hyperthyroidism when administered to patients with adenomatous goiter. The severity of the symptoms of exophthalmic goiter may be almost completely relieved and the basal metabolism may be decreased by administration of iodine. The iodine appears to exert its action through the thyroid gland rather than through the tissues of the body. Iodine does not affect the physiological activity of thyroxine in the normal experimental animal.

Chapter 18

Clinical Aspects of Thyroid Disease

It is significant that the disease, exophthalmic goiter, is practically unknown except in the human being. For this reason it is obvious that an exhaustive study of the clinical conditions associated with various types of hyperthyroidism and hypothyroidism in man furnishes the complete panorama of the influence of the thyroid gland. Plummer [400] has stated that there are three clear-cut, abnormal, physiologic states associated with anatomic changes in the thyroid: "pure" hypothyroidism, "pure" hyperthyroidism, and that of exophthalmic goiter.

HYPOTHYROIDISM

The condition known as myxedema is attended with a complex clinical syndrome. Boothby [60] describes the clinical condition of myxedema as follows: "The onset is slow and gradual. There is a feeling of lassitude, lack of energy and a general slowing of activities usually accompanied by some increase in weight with decrease in appetite and the development of a hard, non-pitting edema. The skin feels thick, dry and scaly, particularly over the hands, arms and legs. On examination fine scales can be seen on the inside of the stockings. The nails are often striated and brittle. There is a marked decrease and often entire absence of perspiration. The hair becomes dry; it is more often fine than coarse and some patients become quite bald. The eyelashes and eyebrows may fall out, the outer thirds being usually first affected. The features are broad, coarse and mask-like with thick lips and nostrils and puffiness around the eyes. While most noticeable in the face there is this same subcutaneous, non-pitting infiltration of the tissues of the hands, feet, supraclavicular fossa and in fact of the whole body. The tongue is large and maneuvered by the patient in a very awkward manner. The speech is slow and thick. The acuity of hearing and vision may be decreased. The sense of smell may be decreased. Perversion of the sense of taste is not infrequent. There is a gradual slowing of the mental processes with forgetfulness which later becomes more marked with the development of symptoms of imperfect cerebration evidenced by delusions and by

hallucinations of hearing or of sight. As a general characteristic these patients are calm, placid and indifferent. Some, however, have a definite irritability and nervousness which may progress to a condition of suspicion almost amounting to a true psychosis or paranoid dementia. Mental effort, like reading or listening to music, is very tiring and cannot be long maintained. Drowsiness and a tendency to drop off to sleep during the daytime are prominent symptoms. The movements are slow and coördination is sometimes slightly impaired. Falling or stumbling is not infrequent. The walk is quite characteristic and may be likened to the waddling gait of a duck. The reflexes may be diminished. The joints and muscles are often painful. Patients are chilly, desire extra clothing and seriously object to room temperatures comfortable for healthy people. All their symptoms are accentuated during cold weather. The oral temperature usually ranges between 97° and 98° Fahrenheit. Some of these patients continue to work for years even while suffering from a high-grade myxedema.

"Cutaneous ecchymoses may occur but are rather rare. Hemorrhage from the mucous membranes is quite common. Bleeding hemorrhoids are especially frequent. Secondary anemia is often a pronounced symptom; occasionally the blood picture resembles that of pernicious anemia resulting in a mistaken diagnosis." It is a remarkable fact that all of these signs and symptoms which are associated with a decrease in the activity of the thyroid gland are promptly relieved not only by the administration of thyroid material, but also by the single substance, thyroxine.

A study of comparative physiology and pathology shows that the nervous system and probably all other systems of organs are more affected by alterations in thyroid activity in man than in any other animal organism. Carlson, Rooks and McKie [99] have investigated the effect of thyroid administration in a series of animals and found that there were great variations. They concluded that man is much more sensitive than any experimental animal tested. Stoland [407] has shown that animals vary greatly in their tolerance to thyroid substance. I pointed out many years ago that the physiological dose of thyroxine which would bring about marked effects in the human being was approximately one seventy-fifth as great as that required in the dog.

"PURE" HYPERTHYROIDISM

Plummer [401] has related the activity of the thyroid to the condition of hyperthyroidism. "The following observations make it seem probable

that the hyperthyroidism associated with adenomatous goiter is due to an excess of the normal active agent of the thyroid in the tissues of the body: (1) the primary phenomena observed are only exaggerations of normal physiologic reactions; (2) from our knowledge of physiology we can conceive of no simpler reactions occurring with an elevation of the basal metabolism; (3) these reactions return to their normal level in three weeks following the enucleation of an adenoma, and (4) the phenomena in no way differ from those following the administration of desiccated thyroid or thyroxine."

EXOPHTHALMIC GOITER

Plummer [401] distinguished hyperthyroidism and exophthalmic goiter as follows: "The complex of hyperfunctioning adenomatous thyroid can be reduced to physiologic terms, hence, can be covered in a few short sentences. That of exophthalmic goiter is fluctuating in character and degree and not as yet wholly reducible to physiologic terms.

"The clinico-physiologic complex of exophthalmic goiter can readily be conceived as that following the administration of thyroxine, or that associated with hyperfunctioning adenomatous thyroid plus certain notable characteristic findings that can be grouped as ocular symptoms, the characteristic nervous phenomena, and the tendency to crises which may terminate in death."

DYSFUNCTION OF THE THYROID

For many years Plummer has believed that the symptoms of exophthalmic goiter are due to dysfunction of the thyroid. For a detailed discussion of the clinical evidence supporting this theory the reader is referred to his publications. Plummer [401] makes the concrete suggestion that in exophthalmic goiter, in addition to the excess thyroxine responsible for the hyperthyroidism, an abnormal substance is produced in the gland. The ratio between the amounts of thyroxine and the abnormal product varies in different individuals and in the same individual from day to day. For this reason the severity of the symptoms in exophthalmic goiter fluctuates markedly: the extreme nervous manifestations in the so-called goiter crisis are apparently due to excessive amounts of the abnormal product.

Janney [252] showed that some cases of hyperthyroidism were improved by thyroid medication and suggested that a premature discharge of a hypothetical, toxic, intermediate product caused the symptoms of exophthalmic goiter. This condition brought about impoverishment of iodine,

and, as a result, the thyroid gland functioned in a condition in which its essential products were limited in amount. Breitner [71] has expressed somewhat the same views so far as loss of storage function is concerned. The question whether or not there is a definite substance which is produced in the gland in exophthalmic goiter and the determination of the functioning groups in such a substance are among the remaining important problems related to the chemistry of the thyroid gland. When the thyroid gland in a case of exophthalmic goiter is removed the patient may be entirely relieved, but the gland may again hypertrophy and all the symptoms recur. In some cases a second operation for the removal of a portion of the gland is necessary and in a few the gland has been resected as often as three times for relief of recurring hyperthyroid symptoms. This is in marked contrast to the rare recurrence of hyperthyroidism after removal of a toxic adenoma (Plummer [402]).

Another significant difference between hyperthyroidism and exophthalmic goiter is the rapidity of onset of symptoms. It requires on the average several years for an adenoma to develop the status necessary to cause toxic symptoms, but a critical case of exophthalmic goiter may develop within a few months (Plummer [401]).

Finally, the two diseases are distinguished by the use of Lugol's solution. The basal metabolic rate and severity of the disease are not diminished when iodine is administered in a case of toxic adenoma, but the relief produced in cases of exophthalmic goiter may be striking.

Kunde [297] has apparently produced exophthalmos by the administration of thyroid material in rabbits which were first thyroidectomized. If the cause of exophthalmos in the rabbit and in man is the same, then it would appear that this unusual condition of the eyeball may be due to the normal constituents of the thyroid, provided other physical conditions are in proper relation to increased thyroid activity. Further work must be done on this subject.

SUMMARY

The disease exophthalmic goiter is practically unknown except in the human being. A detailed clinical study of hypothyroidism, hyperthyroidism and dysthyroidism furnishes the complete panorama of the physiological significance of the thyroid gland. All of the subjective and objective symptoms of myxedema are relieved by thyroxine. This indicates that no other substance is essential for the manifestation of the physiological effects of the thyroid. Hyperthyroidism associated with adenomatous goiter is due to an excessive outpouring of thyroxine. The

symptoms of hyperthyroidism differ in no way from those following the administration of desiccated thyroid or thyroxine. Exophthalmic goiter is characterized by nervous phenomena and by a tendency to terminate in the so-called goiter crisis and death; the clinical symptoms in exophthalmic goiter suggest that this disease is due to both hyperthyroidism and a dysthyroidism.

Chapter 19

The Function of the Thyroid

"The thyroid gland performs its function so silently that but little regarding this function has been learned from the study of normal persons" (Plummer [898]).

Before any experimental data had been secured concerning the physiological effect of the thyroid secretion, speculation concerning the function of the gland was, of course, confined to the gland itself. It is interesting to note the gradual alteration in the viewpoint concerning the function of the thyroid gland as our knowledge of this organ and of its significance in physiology has increased.

Meuli, in 1883, suggested that the function of the thyroid gland was to act as a blood reservoir and blood regulator. When too much blood was passing to the brain the thyroid enlarged, compressed the carotid and prevented an oversupply passing upward; when too little blood reached the brain the thyroid decreased in size and relieved pressure on the carotid. Goiter was more frequently seen in women because of the greater circulatory demands. Proper exercise, especially when begun at an early age, would reduce the size of the thyroid gland or goiter. This was particularly recommended for persons living in the mountains where more demands were made on the heart and the circulatory apparatus. Meuli states that the following theories had been offered up to 1883, concerning the function of the thyroid gland: (a) the thyroid is a blood regulator for the upper half of the body, especially the brain; (b) it has the function in part of a blood-forming, in part of an ordinary gland; (c) the thyroid is an accessory organ for the formation of the voice; (d) it is an organ of respiration; (e) it is the middle point of the capillary system; (f) the thyroid has the purely mechanical function of an elastic pad.

Research has slowly enlarged the circle which circumscribes those physiological processes recognized to be under the regulation of the thyroid gland and today it can be clearly stated that the gland determines the pace which is to be maintained by all of the other organs and systems

of organs in the body. The function of the thyroid gland may be regarded as twofold: first, the synthesis of thyroxine in its "active" form, and second, the regulation of the velocity of physiological processes through the action of thyroxine. Alteration in the capacity of the gland to synthesize thyroxine may result only in an enlargement of the gland such as is seen in endemic goiter. Alteration in the amount of active thyroxine which is discharged from the gland brings about systemic changes which eventually affect all bodily functions.

THE DETOXICATING FUNCTION OF THE THYROID

When Baumann separated iodothyrin from the thyroid he did not hesitate to suggest that it elaborated a secretion which was essential for the maintenance of the normal physiological status of the body and that the iodine compound which was contained in iodothyrin represented the essential constituent of the gland. In opposition to this view, Notkin [369] believed that the thyroid gland possessed a detoxicating function and that the thyroid proteins did not contain a secretion of the gland.

Wormser [530] agreed with Notkin and suggested that the thyroid gland acted as a purifying organ. Its duty was to separate toxic products, of protein nature, circulating in the blood, and to remove from circulation other substances with which the toxic protein compounds were neutralized. He suggested that iodine served for the preparation of an antitoxin made by the gland. If too small an amount of iodine was present, toxins accumulated in the gland and eventually produced goiter. Although iodine functioned as an aid in the detoxication processes, iodine and iodothyrin were not of themselves sufficient. He also believed it was necessary to have present the whole gland substance.

The detoxicating theory of the function of the thyroid gland received its strongest support from Blum,[47, 48, 49, 50, 51] who believed that the organ did not have a secretion which was poured into the blood stream or lymph, and that its action was entirely one of detoxication of the poisonous products which were brought into the gland through the blood stream. He believed that the iodine compounds enabled the thyroid to carry out this essential action, and suggested that some of the products probably originated in the intestine. In the absence of normal thyroid activity the accumulation of toxic products produced goiter and the symptoms of hypothyroidism. Blum believed that the iodized proteins of the thyroid gland were toxic when present in abnormal amount and that over-production of the essential compound brought about hyperthyroid symptoms.

The principal experimental result on which Blum based the theory that the detoxicating action of the gland took place within the gland itself was the failure to find any iodine in the blood or lymph from the thyroid gland. From this negative result Blum concluded that the thyroid did not discharge its iodized protein. In a continuation of the investigation Blum and Grützner [54] showed that the iodine content of the dog's thyroid was not constant and that the iodine was in combination with protein in the gland. The gland was able to increase its content of iodine after the administration of potassium iodide, and after this had occurred the gland guarded its increased store of iodine for a long time. This they believed was evidence that the detoxicating action occurred in the thyroid gland itself and this rendered still more improbable the theory that the function of the thyroid was to prepare an iodine-containing secretion.

In 1920, Blum and Grützner [55] showed that iodothyreoglobulin was in part decomposed after it had been injected into the circulation of a dog. The same action occurred in thyroidectomized animals. Thus the decomposition of the iodized proteins of the thyroid was not due to the thyroid gland. It was shown that only the liver had definite power to destroy the iodized proteins of the thyroid. This action was demonstrated by perfusion of liver with dog's blood containing an extract of the thyroid. The liver rendered acetone-soluble some iodine compounds which were originally insoluble in acetone; furthermore a small amount of iodide-ion was also formed. Blum and Grützner [55] again expressed the view that the activity of the thyroid gland depended on absorption of certain substances from the circulation by the gland rather than through the discharge of an iodized protein into the blood and lymph.

In 1927, Blum [52] again investigated the problems concerning the iodine content of the blood and the significance of iodine in the thyroid gland. He criticized colorimetric methods and concluded that the method of Blum and Grützner is the only one which is satisfactory for the determination of iodine in animal tissues. This method, however, is limited to the determination of amounts of iodine greater than 0.004 mg. He stated that the best colorimetric method was no more sensitive than this. He repeated the former experiments of Blum and Grützner and found that if an animal had been fasted for one month, the blood did not contain iodine, although the thyroid gland contained significant amounts.

He suggested that through the action of an agent, "iodase," the thyroid can split potassium iodide and build the iodine into a protein complex. In addition to this action, he believed that there is a second

characteristic property of the normal thyroid, the ability to securely retain this iodized protein within the gland.

In goiter, the thyroid gland might lose its power to fix the iodine or under other conditions it could build the iodine into the protein complex but could not retain it. Administration of iodine was of therapeutic value only in case the gland could utilize the iodine for the formation of iodothyreoglobulin. In conclusion, Blum stated that neither chemical nor biological investigations show the presence of an iodized protein in the blood derived from the thyroid gland.

The contributions of Blum and coworkers have extended over many years and must be carefully considered; however, it is apparent that the interpretation of the activity and function of the thyroid has been largely influenced by the determination of iodine in the gland and tissues of the body. At the time of their first attempt to prove the presence of iodine in the blood and lymph no satisfactory method was available and the method which Blum and Grützner [53] devised was not adequate. Kendall and Richardson and Veil and Sturm [515] have shown that the total amount of iodine in the circulating blood of an adult human being is not more than 0.8 mg., and even with the most refined methods it has not yet been proved that iodized proteins are discharged from the thyroid gland during stimulation of the thyroid nerves. It has been shown, however, that the iodine content of the blood does vary from season to season, and there can be no doubt that some equilibrium exists between the thyroxine in the thyroid gland, and that in the tissues.

The evidence submitted by Blum and Grützner concerning the stability of the iodized proteins in the thyroid was incorrectly interpreted. They assumed that the essential iodine compound of the thyroid was destroyed by the liver. Thyroxine is not destroyed and but a small amount if any of its iodine content is excreted as iodide in the urine. If injected in excessive amount a portion of it is excreted in the bile, but it has been shown to be remarkably stable in the animal organism. Although a large amount of the injected thyroxine may be excreted in the bile, the portion which is incorporated in the active form is sufficient to bring about the essential physiological effect of thyroid material. It is incorrect to assume that the iodine-containing active constituent of the thyroid is completely destroyed by the liver and cannot function outside of the thyroid gland. The results of Blum and Grützner are probably explained by the presence of several iodine-containing compounds in the thyroid; some are decomposed in the liver but sufficient of the essential substance, thyroxine, remains to account fully for the activity of the

thyroid material used. This result is also manifested by thyroidectomized animals.

These results make it apparent that Blum's theory rests upon evidence which is essentially negative in character: first, the failure to demonstrate the presence of iodized protein in the blood and tissues, and second, the failure to recognize the stability of thyroxine and the extraordinarily small amount necessary to produce the physiological response which is typical of thyroid material. The best answer to Blum's theory concerning the detoxicating action of the thyroid and to the question as to whether or not it elaborates an essential secretion is given by a study of the physiological effects of pure, crystalline thyroxine. Since this compound will relieve all of the symptoms of hypothyroidism and also will produce all of the effects which are sequels to the oral administration of desiccated thyroid or of fresh thyroid glands, the hypothesis that the thyroid gland is a detoxicating agent for toxic substances in the blood stream and that it does not produce a secretion, which functions outside of the thyroid gland, becomes untenable.

A more recent contribution concerning the detoxicating action of the thyroid gland itself, in contradistinction to the elaboration of an essential constituent which functions in the tissues and not in the gland, has been put forward by Starlinger.[463] He found marked changes in the blood proteins, especially fibrinogen, associated with variations in thyroid activity, and believed that the function of the thyroid was made manifest by the changes brought about in the blood proteins. He [464] suggested that the function of the thyroid was to decompose high molecular proteins and to give up protein split products into the blood; the specific action of the gland was brought about by an agent catalytic in nature which does not leave the gland. A specific secretion in the strict sense of the word was denied. Over-development of the normal function of the thyroid gland was suggested as the cause of goiter. It is difficult to reconcile the known effect of thyroxine on the basal metabolic rate with this theory.

THE NATURE OF IODOTHYREOGLOBULIN AND THYROXINE

Oswald has made many contributions to our knowledge of the thyroid. His work has been chiefly concerned with the chemical nature and the physiological activity of the iodine compound which occurs in the gland. Oswald has not agreed with the detoxicating theory of Blum; on the other hand, he has felt ever since the separation of iodothyrin by Baumann that this compound did not represent the active constituent of

the thyroid. For many years he has championed iodothyreoglobulin as the active constituent of the thyroid and, although he has admitted that iodothyreoglobulin is too complex to furnish any insight into the essential chemical nature of the iodine compound, his work on the hydrolysis of this substance has led him to believe that if iodothyreoglobulin is altered by any hydrolytic agents its essential properties are destroyed.

Oswald [378, 382, 383, 384, 387, 388, 389] has reported many investigations of the function of the thyroid, a few of which will be reviewed as they represent definite progress in the interpretation of the function of this gland. He [385] first showed that there was an apparent increase in the action of certain drugs after injections of iodothyreoglobulin. Furthermore, the response of the nerves to stimulation [386] appeared to be increased after injection of iodothyreoglobulin and he concluded that this compound possessed the ability to increase nerve tone "in an exquisite manner." These experimental results suggested that in man the severity of exophthalmic goiter was dependent on the constitutional status of the individual with hyperthyroidism. The condition of "altered nerves," hysteria, epilepsy, pronounced neurasthenia, or congenital defects in respect to the nervous system all caused a difference in the symptoms of hyperthyroidism. In 1925, Oswald [389] again reviewed the relation between the thyroid gland and the nervous system. He discussed the probable nerve stimulation of the thyroid gland and pointed out that the thyroid secretion itself increased the response of a nerve to stimulation; therefore its action was interpolated between the nerve endings and the affected organs. He assumed that the gland was stimulated by the nerve centers and, since the discharge of its secretion increased the action of these centers, it acted as a multiplier of the nerve stimulus. In this respect the thyroid differed from the suprarenal and the pituitary glands. The stimulatory threshold of a nerve may be high or low and the response of any given individual to thyroid medication was determined by this threshold.

Oswald believed that iodothyreoglobulin possessed the full physiological activity of the thyroid gland and that thyroxine did not affect the nerves which control the circulation, that is the vagus and splanchnic, in the same way as iodothyreoglobulin. He referred to the work of Hammett,[190] who showed that thyroxine failed to affect the rhythmic beats of a strip of intestine as does the expressed juice of the thyroid gland. Finally, Oswald suggested that thyroxine was not the complete hormone of the thyroid and may not exist as such in the thyroid gland.

The view of Oswald [389] concerning thyroxine and the physiological

activity of the thyroid gland may be summarized under the three heads: first, the physiological effect of fresh thyroid juice; second, the value of "acute" experiments for the determination of the physiological properties of the thyroid; and third, the activity of thyroxine in acute and long-continued experiments. It is true that the expressed juice of the thyroid gland brings about an effect on the strip of intestine and on other physiological processes which are not given by desiccated thyroid, iodothyreoglobulin, thyroxine or any other thyroid preparation. This effect may be explained by the presence of "active" thyroxine which is present in the thyroid secretion in a form suitable for immediate utilization, or it may be due to some substance not specific to the thyroid gland, such as choline, which is apparently the agent that produces a drop in blood pressure when extracts of the thyroid gland are injected (von Fürth and Schwarz [166]).

When the thyroid gland is investigated by means of acute experiments, the results are open to question. Practically all of the older work of Oswald, Kraus and Friedenthal, Asher and Flack, von Cyon and many others has been somewhat shaken if not discounted by the newer work in which fluid electrodes have been used and more careful control of the hydrogen-ion concentration of the blood has been carried out. A slight increase in the pH of the blood has been shown to produce a marked change in the physiological effect produced by epinephrine, and undoubtedly other substances which have been investigated by acute experiments are influenced by similar changes in the acid-base equilibrium. However, it is easily shown that after an injection of thyroxine there is no immediate response of any kind. This observation excludes the acute type of experiment for the study of the physiological activity of this compound.

If thyroxine is administered in proper doses for a sufficient length of time it will bring about every effect which follows an injection of iodothyreoglobulin. It is therefore necessary to assume that after thyroxine has been injected into the animal organism it becomes incorporated and activated in the functioning protoplasm and that, when in this condition, it is the full equivalent not only of iodothyreoglobulin but of the fresh juice of the thyroid gland so far as all symptoms of hypothyroidism and hyperthyroidism are concerned.

In regard to the formation of thyroxine from some other substance one may say without hesitation that it is impossible to elaborate a compound such as thyroxine from any other substance by means of hydrolysis with sodium or barium hydroxide, and it seems highly probable

that thyroxine must exist as such in the thyroid gland, except for the bond which holds it within the protein molecule. This linkage, however, probably involves only the carboxyl or the amine group, or both.

DOES THYROXINE HAVE A SPECIFIC ACTION?

Simpson [453] questions whether thyroidectomy is the primary cause of the arrested development of skeletal muscle in cretin sheep. She suggested that the lack of development might be due to lethargy rather than to a specific action of the thyroid on growth. From the work of Hoskins [233] concerning the influence of the acetyl of thyroxine on the growth of certain parts of the body, notably the bones, and from the results of Kunde and Williams on the influence of thyroidectomy on the growth of the bones, it seems probable that the question asked by Simpson must be answered in the affirmative. Anything which will cause the thyroid gland to enter into a state of inactivity may bring about a similar condition in the animal organism, but the cessation of growth of the long bones and changes in the epiphysis appear to be directly influenced by thyroxine; it is difficult to escape the conclusion that thyroxine has a direct and specific effect on certain phases of the development of the structures in the body. This view is strengthened by the marked influence of thyroxine and of many other iodine compounds on the metamorphosis of the amphibian larvae which must be regarded quite separately from its effect on the basal metabolic rate.

Other evidence of a direct and specific effect of thyroxine on growth is furnished by a study of hairless pigs. This condition has been shown to be due to an alteration in the functional capacity of the thyroid gland and is relieved either by a change in diet or by administration of iodine.

The large amount of work which has been carried out may be summarized in the statement that there are apparently two distinct effects of thyroxine. One is on the basal metabolic rate; this is unique for the substance, thyroxine. The other effect is on the growth of the bones and skin and the metamorphosis of the tadpole; this physiological response is produced not only by thyroxine, but by the acetyl of thyroxine, and in the metamorphosis of the tadpole by many other substances.

THE FUNCTION OF THE THYROID GLAND AS DEDUCED FROM CLINICAL STUDY

Although the entire scope of thyroid activity becomes apparent through a study of the clinical conditions of hypothyroidism, hyperthyroidism and dysthyroidism, but few clinicians have succeeded in analyzing the confusing mass of clinical results into a working hypothesis

which expresses the function of the thyroid gland. A study of the prevalence and the severity of the symptoms establishes beyond any question the fact that the clinical manifestations associated with various types of goiter are largely influenced by geographical distribution and by racial characteristics. Aschoff found that hyperthyroidism in different parts of Germany differs within a wide range, and a review of the clinical study of the types of goiter in the United States and Europe, particularly in Switzerland, shows a wide divergence in the clinical manifestations of hyperthyroidism. Plummer [401] has concluded that the severity of the symptoms of exophthalmic goiter in the United States is largely dependent on the section of the country in which the patient resides. This variation was evidently related to the iodine available in the food supply.

Probably nowhere are there more favorable conditions for the study of hypothyroidism and hyperthyroidism than in the Mississippi Valley. Here hyperthyroidism is seen in a most severe form and the disease myxedema cannot be described as rare. Through a detailed study of by far the largest group of patients that have been investigated, Plummer [400] has analyzed the normal and abnormal influence of the thyroid gland and has formulated a theory in regard to the activity of this gland. He [401] believes that the thyroid secretion functions throughout the cells of the body and does not primarily influence any one organ or system of organs. He has concluded that it does not, of itself, directly cause the production of energy, but that it raises the capacity of any given cell to respond to excitation or stimulation. He has suggested the following concise expression for the function of thyroxine: "Thyroxine is a catalytic agent hastening the rate of formation of a quantum of potential energy available for transformation on excitation of the cells." While this definition is given in broad terms it nevertheless furnishes a viewpoint which can be more specifically defined in any detail. It is a summary of what is known of the physiology of the thyroid which has not yet been supplanted by a more exact definition expressed in terms of chemistry.

In regard to the thyroid gland itself, Plummer [400] believes that the function of the gland is to make thyroxine and colloid, which is an accessory to the production and storage of thyroxine and iodine used in the elaboration of this compound. Plummer has concluded that there is no definite evidence that the thyroid has any other function.

OTHER FUNCTIONS ASSIGNED TO THE THYROID GLAND

The number of functions which have been assigned to the thyroid gland is legion: a few hypotheses will be reviewed. von Cyon [117, 118] showed that although iodine had an effect on the nerves of the heart and blood vessels, injection of iodothyrin produced the opposite effect. From this he concluded that one function of the thyroid gland is to make iodothyrin and thereby protect the body from the action of iodine.

Kocher [283] pointed out that one function of the thyroid gland was concerned with the formation of the various cellular constituents of the blood. He showed that tincture of iodine changed the blood-cell count and suggested that some iodine-containing substances in the thyroid gland brought about certain changes in the blood: other substances influenced favorably this action of the iodine-containing proteins of the gland. Kocher found that greater changes in the blood cells were brought about by iodothyreoglobulin than by iodothyrin.

Fonio showed that in myxedema changes in blood cells and a low hemoglobin content were frequently present. Administration of thyroid material, which contained iodine, relieved the anemia and the blood-cell count returned to normal. Lanz found that the blood-cell count was affected by administration of iodothyrin and iodothyreoglobulin. An increase in the hemoglobin content of the blood has been noted in many cases of myxedema which have been treated with thyroxine by Plummer and Boothby.

Herzfeld and Klinger [217] suggested that the secretion of the thyroid could not be a protein body and must be a lower product of protein hydrolysis. They quoted the work of Abelin [9] and of Eiger [141] with iodine-free preparations which apparently showed physiological activity which could not be explained by the presence of protein. Herzfeld and Klinger believed that goiter degeneration was due to insufficient autolysis of the proteins in the thyroid cells, and that the iodine was present as a salt combination of protein split products. They regarded iodine as an unimportant constituent, and suggested that its function was to promote the formation and excretion of the thyroid secretion. Stimulation of the nerves to the thyroid gland was also believed to increase the hydrolytic processes in the cells. It seems probable that this hypothesis, which was suggested in 1918, will not stand the test of time, and certainly the view regarding the significance of iodine cannot be maintained even today.

Timofejewa has shown that there was a relationship between the thyroid function and blood catalase. This relationship did not appear to

depend directly on iodine, the iodide-ion or iodized proteins: it was related to processes of oxidation.

Tsukamoto [502] has studied the utilization of oxygen by the blood. Defibrinated blood was saturated with oxygen, and placed in cylinders under oil. A series of ten 1.5 cc. samples was held at 37°. Every two to three hours two tubes were removed and the oxygen was determined in the Barcroft apparatus. The blood of the rabbits which had been fed thyroid material showed increased utilization of oxygen. The blood of thyroidectomized rabbits showed less utilization of oxygen than the normal. The blood of exophthalmic goiter patients showed increased utilization of oxygen. The variations in the utilization of oxygen were traced back to possible variations in the thyroid constituents present in the blood.

Janney [253] has suggested an anabolic function of the thyroid gland and thyroxine. He previously had shown that, although administration of large amounts of desiccated thyroid or thyroxine brought about increased elimination of nitrogen, the administration of minimal doses in the condition of cretinism was followed by the retention of nitrogen, and by marked increase in body weight. He therefore suggested that the thyroid gland possessed an anabolic function as well as a catabolic. Although this could not be stated in chemical terms, in a manner which could be proved, he believed that the elaboration of carbon compounds was facilitated or brought about by the action of thyroxine.

Fenger [152] investigated the relation between the thyroid and the suprarenal glands in the life of a calf fetus. He was able to show that the thyroid gland contained iodine and the suprarenal glands contained epinephrine in the fetus, although the digestive glands were not active. The pancreas of a full-grown hog showed diastatic power 140 times greater than the pancreas of the fetus, while the thyroid gland of a pregnant hog contained only one-half to one-third more iodine than the corresponding fetus. He suggested that both the thyroid and suprarenals of the fetus take a distinct and active part in growth and development of the unborn animal.

THE RELATION TO IMMUNE BODIES

Many investigators, among whom may be mentioned Fjeldstad, Ecker and Goldblatt, Garibaldi, Lanzenberg and Képinow, Houssay and Sordelli,[234, 235] and Clevers have endeavored to relate the formation of antibodies with the action of the thyroid gland. Koopman questions whether endocrinology may not open to serologists and pathologists a

wider entrance for the investigation of the field of antibody formation. Marine,[336] however, concludes that "at present the results obtained do not warrant any direct association of the thyroid with antibody formation. The reaction to infections, as shown by a reduction in iodine store and a tendency to hypertrophy and hyperplasia, clearly indicate the thyroid is an important indirect factor in resistance to infections."

THE RELATION TO METHYLATION

Stuber and Stuber, Russman and Proebsting have suggested that the thyroid gland was concerned with the processes of methylation in the body. This hypothesis was elaborated from the suggestion that one method for the addition of alkyl groups to amines is through the use of alkyl iodides, and, since the thyroid secretion contained iodine compounds, it seemed probable that alkylation induced by thyroid activity might be an index of its power. This was investigated by the determination of the ability of rabbits to methylate guanidine acetic acid. It was shown that after thyroidectomy these animals lost the ability to methylate guanidine acetic acid; feeding thyroid material restored this ability. It was also found that the administration of iodine or the blood of normal animals to the thyroidectomized rabbits restored the ability to methylate. They believed that methylation of guanidine acetic acid was bound up with the function of the thyroid gland in rabbits; however, they pointed out that this was only one phase of the function of the thyroid.

INFLUENCE OF ENVIRONMENT

The general systemic disturbances brought about by removal of the thyroid or through administration of thyroid material, have been carefully studied. The results afford a summation of the alterations produced in the animal organism by a deficiency or excess of thyroid substance. The function of this ductless gland has also been studied by the determination of the effect of changes in the diet. Inanition and a deficient type of diet may produce atrophy; over-feeding, particularly of protein, may produce hypertrophy of the gland. In addition to these influences still another important factor has been determined: temperature has a marked effect on the activity of the thyroid gland.

Mills [354] and others have shown that a high external temperature caused diminished activity of the thyroid gland, as judged by morphological changes, together with a coincident decrease in the rate of growth. Stoland and Kinney have shown that desiccated thyroid is less toxic to rats when the temperature is increased.

Sugata has extended the work of Mills and has shown that both quinine and a high external temperature caused an increase in the iodine content of the thyroid gland; starvation caused a decrease. One lobe of the thyroid gland was analyzed at the beginning of the experiment and the second lobe after the changes had been brought about by the various treatments.

Fenger [154] has suggested that the temperature factor is the most important of all in producing seasonal variations of the iodine content of the thyroid gland. The marked variations found by Kendall and Simonsen [275] in the iodine and thyroxine content, which have been reported in this monograph, confirm the work and strengthen the conclusions of Fenger.

Riddle [414, 415] has carried out an extensive investigation concerning the seasonal changes in the thyroids of doves. He found a marked change in the weight and iodine content of the thyroid gland of doves with the changes in the season. After a careful review of the literature and a discussion of the work which has been done in this field, Riddle and Fisher presented evidence that conclusively showed a close relation between variations in the size and apparent function of the thyroid gland associated with seasonal changes and the activity of other glands in the body.

Still other evidence which relates the activity of the thyroid gland to the temperature and activity of the animal organism has been presented by Adler,[15] who found that during hibernation animals showed regression and atrophy of the thyroid gland, although in the summer the gland of the same animal had a normal appearance. Injection of thyroid extract into a hibernating hedgehog brought about an increase in temperature and rate of respiration and the animal resumed his normal activity. When physiological salt solution was injected there was no influence on the hibernation. Extracts of the thymus, epinephrine, proteinogenous amines, phenyl and oxyphenylethylamine brought about the same effect as thyroid injections. Extracts of the pancreas, epiphysis and breast produced no effect. The influence of the hypophysis was questionable. Antipyrine did not prevent the action of thyroid extract on the animal during hibernation, but quinine did prevent it. Marin could not show significant changes in the thyroid associated with hibernation of the spermophile. Thyroidectomy did not alter the course of hibernation.

SUMMARY

Theories regarding the function of the thyroid gland were first concerned with the probable mechanical influence of the gland on the rate of the blood flow, and so forth; later it was recognized that the gland exerted an influence throughout the various tissues and systems of organs. Iodothyrin was believed to be the essential constituent of the secretion of the thyroid which was delivered from the gland to the blood stream. An alternative hypothesis suggested that the gland acted as a detoxicating agent but that it did not elaborate a secretion which functioned outside of the gland. Iodine was assumed to assist the thyroid in its detoxicating action on toxins removed by the gland from the blood.

Oswald believed that the thyroid prepared a secretion and that iodothyreoglobulin was the essential constituent. He suggested that any hydrolysis of iodothyreoglobulin, either with acid, alkali or enzymes destroyed its essential physiological activity. Thyroxine will produce every effect of iodothyreoglobulin. It cannot be assumed that thyroxine is elaborated by hydrolysis with alkali from any less complex precursor which exists in the gland.

The excitability of the nerves as it was first measured in a study of the activity of iodothyrin and iodothyreoglobulin is not a satisfactory criterion. Some physiologically active substance may be present in the thyroid gland which is not specific for this gland. All of the effects of fresh thyroid juice may be due to minute traces of "active" thyroxine.

A clinical study of thyroid activity has suggested that thyroxine is a catalytic agent which functions throughout all the cells of the body.

Many hypotheses have been offered concerning the influence of the thyroid on the formation of the various cellular constituents of the blood, immune bodies, methylation in the body, blood catalase, and anabolic reactions. It has been shown that the thyroid gland is less active in a high external temperature: thyroid material is more toxic in a low external temperature. The gland appears to vary in its histological appearance with seasons of the year and in some animals with hibernation.

Chapter 20

Chemical Reactions Which Are Influenced by Thyroxine

When Baumann discovered that iodine was a normal constituent of the thyroid gland knowledge concerning the chemical nature of the essential iodine-containing compound present in the gland and the physiological action of other iodine compounds was insufficient to furnish more than the barest outline of how the thyroid gland brought about its effect in the animal organism. Moreover, nothing was known of the chemical nature of the active constituent of any of the other ductless glands, nor of the amount of material required to produce a measurable effect. The rate at which thyroxine is destroyed and the rate at which epinephrine is poured into the blood stream were unknown quantities. Changes were shown to occur in the rate of many physiological processes, in the distribution of water in the body, and in so many other constituents that it was extremely difficult to determine which reactions were directly and which were indirectly influenced by thyroid activity. It was for these reasons that the detoxicating theory of Notkin, Wormser, and Blum and others could not be answered with incontrovertible evidence.

This difficulty is well illustrated by the interpretation which was applied to the action of the thyroid gland on nitrogen metabolism and on the basal metabolic rate. Even before iodine was discovered as a normal constituent of the thyroid gland it had been shown that nitrogen metabolism was influenced by administration of thyroid material.

The results of a large number of investigations concerning the relationship between the thyroid and nitrogen metabolism have now shown that it is impossible to change the concentration of thyroxine functioning in the animal organism without causing a corresponding change in the protein metabolism. This is an established fact, but this knowledge does not furnish an explanation of the mechanism by which the thyroid is able to bring about this result.

Lusk,[317] in 1913, submitted evidence which showed that there was an

increase in the heat production associated with phlorizin glycosuria. If the thyroid gland was removed the increase in heat production did not occur. Lusk concluded that the reason for this was that protein metabolism did not increase and therefore did not afford the stimulus to metabolism through the action of amino acids. This view of thyroid activity placed the effect of the thyroid on the basal metabolic rate secondary to its effect on the nitrogen metabolism.

Contrary to this conception of the action of the thyroid Deuel, Sandiford, Sandiford and Boothby [124] have shown that, although protein metabolism was temporarily increased by the administration of thyroxine, still the prolonged presence of abnormally large amounts of thyroxine in the animal organism did not result in a sustained increase in protein metabolism. This result at once assigns a primary position to the effect of the thyroid on oxygen consumption and the temporarily increased nitrogen elimination after an increase in thyroid activity as a secondary reaction.

The one constant observation concerning changes in the velocity of chemical reactions which are produced in the animal organism by the administration of thyroid material or thyroxine is the change in the consumption of oxygen and the output of carbon dioxide. Whenever the concentration of thyroxine which is active in the tissues is changed the output of carbon dioxide is changed. This has been known since the work of Magnus-Levy, in 1895, and since 1917 it has been known that pure, crystalline thyroxine alone will produce the same reaction.

It has been suggested that the action of the thyroid gland resembled the effect of a bellows on a fire. Plummer has compared the thyroid to the cylinder of an engine of internal combustion. The conclusion can be safely drawn that the primary reaction, which is altered by a change in the concentration of thyroxine in the tissues, is intimately associated with an increase in the consumption of oxygen and the output of carbon dioxide. The immediate problem, therefore, can be reduced to the question of how the thyroxine molecule can cause an increase in the velocity of the consumption of oxygen and the output of carbon dioxide.

Before the structural formula of thyroxine was known it was impossible to do more than suggest that the thyroxine molecule was affected by oxidation-reduction processes and in turn was able to influence the velocity of the oxidative processes occurring in the animal organism. I made this suggestion several years ago and it was a curious coincidence that the oxindolepropionic acid series of compounds, which were prepared synthetically in the belief that one member of this group of

compounds when iodized would prove to be thyroxine, possessed chemical properties which permitted their oxidation by dyes which have an intensity of oxidation of the same order as that which exists within the tissues. This fact has led to an extended investigation of the oxidation-reduction potentials of oxindolepropionic acid and its derivatives, cysteine, glutathione, epinephrine and related compounds, thyroxine and its derivatives.

The most significant problem now associated with the chemical reactions which are affected by thyroxine is concerned with the question as to whether or not this substance can exist in such a form that it can be oxidized and reduced and whether or not these two forms are involved in the reaction mechanism which modifies the velocity of oxidative processes within the body. The isolation of epinephrine in pure, crystalline form, followed by its analysis and synthesis, has furnished incontrovertible evidence that hydroxyl groups attached to the benzene ring are of great significance in the physiological activity of this compound, and the ease with which epinephrine is oxidized, even by molecular oxygen, suggested that oxidation-reduction processes are involved when this substance brings about its normal physiological response.

OXIDATION-REDUCTION POTENTIALS

The chemical characteristics of compounds which are revealed by a study of their influence on oxidation-reduction potentials have furnished valuable information about many substances which affect biological processes. Reference may be made to the recent investigations of Conant and Scott on hemoglobin and the work of Dixon and coworkers [130, 131] on glutathione.

In order to secure information concerning the action of hydroxyl derivatives of benzene in oxidation-reduction processes, a series of compounds related to epinephrine, was examined by Kendall and Witzemann in regard to their oxidation-reduction potentials. This work can be briefly summarized in the statement that several substances in a series of compounds related to adrenalone have been shown to exist in oxidized and reduced forms. This service of compounds is sensitive to oxidizing agents and they are easily destroyed. Under certain limited conditions it is possible to modify the velocity of the oxidation of adrenalone when epinephrine is present in the solution: adrenalone is not at all oxidized by molecular oxygen unless epinephrine is also present. Whether or not epinephrine, when acting in this manner, actually exists alternately in oxidized and reduced forms is not known; however, it has been shown

that the hydroxyl groups in adrenalone are the portion of the molecule which is affected by oxidizing dyes, and this is presumptive evidence that oxidation and reduction of epinephrine may be intimately associated with the physiological effect of this compound. Roest has shown that phenylene di-amine is oxidized by molecular oxygen in the presence of epinephrine. The recent work of Pugh and Raper has shown a relation between hydroxyl and quinone groups to oxidation of amino acids in the presence of tyrosinase and oxygen.

Another significant factor which has been established in the study of oxidation-reduction potentials of epinephrine and glutathione is activation of the oxidizing and reducing groups. Kendall and Nord have shown that the sulfydryl group of reduced glutathione and the disulfide linkage in oxidized glutathione are relatively stable. Reduced glutathione and cysteine cannot reduce indigo carmine, and the corresponding disulfides cannot oxidize reduced indigo unless there is also present in the solution another compound which activates the -SH and -SS groups. The chemical nature of this activating agent has not been conclusively shown, but it is formed by the action of hydrogen peroxide or sodium disulfide on indigo disulfonate. In the presence of minimal amounts of the activating substance the velocity of reaction is proportional to the concentration of this agent: the chemical reactivity of these sulfur compounds is dependent on the presence of an activating compound. Similar results have been obtained with aldehydes and certain amines. Formaldehyde and other aldehydes can be present in a solution of indigo white in a phosphate buffer pH 7.4 without completely reacting. If to the solution, aniline, methyl or dimethyl amine, alanine, glycine, glutamic acid or cystine is added, the aldehyde and indigo white rapidly react with removal of all reduced indigo from the solution. These reactions constitute conclusive evidence that the activity of the aldehyde is dependent on the presence of other chemical groupings. Similarly, the oxidation of many of the substances in the series of compounds related to epinephrine may be shown to be dependent on the presence of other chemical groupings. Dimethylamino-acetopyrocatechol is not oxidized by methylene blue; it is rapidly oxidized by a dye which has a much weaker oxidizing intensity, indigo disulfonate. Adrenalone is not oxidized by molecular oxygen nor hydrogen peroxide; it is rapidly oxidized by indigo disulfonate. Adrenalone is oxidized with molecular oxygen in the presence of epinephrine much more rapidly when a third substance is also present in the solution. This substance is prepared by the action of alkali on glucose in the presence of molecular oxygen and indigo and, while its chemical

nature is not known, its influence on the oxidation of adrenalone and epinephrine with molecular oxygen is conclusively shown by the determination of the oxidizing compounds present in the solution after oxidation with molecular oxygen.

The specific influence of certain chemical groupings cannot be predicted by the usual thermodynamic equations which have been derived for the velocity and extent of reaction. The mechanism appears to involve the formation of an addition complex: this results in the activation of the reducing or oxidizing agents. Kodama had previously shown that similar results might be obtained with hypoxanthine and its oxidase or with Schardinger's enzyme and aldehyde. Bernheim and Dixon have shown a high degree of specificity for the reduction of nitrates in animal tissues.

It was therefore of peculiar interest to determine the oxidizing and reducing power of thyroxine in order to see whether this substance is easily influenced by an intensity of oxidation which may exist in the animal organism. That thyroxine could be easily oxidized seemed improbable because it is stable in the presence of strong sodium hydroxide and is not affected within a short time by either molecular oxygen or dilute hydrogen peroxide. On the other hand, a solution of thyroxine in phosphate buffer with a hydrogen-ion concentration approximately that of the body, decomposes rather easily and, from the color of the solution, it is apparent that chemical changes, which possibly involve oxidation, can occur.

In order to determine quantitatively the influence of oxidizing dyes on thyroxine, this compound was dissolved in dilute sodium hydroxide and was added to phosphate buffer pH 7.4. It was found to be entirely stable to indigo disulfonate, methylene blue and dibromophenolindophenol. This observation is in keeping with the hypothesis that before thyroxine can bring about its typical physiological action it must be altered, possibly by the addition of some group such as hydroxyl. Other evidence which strongly supports this view is the fact that many samples of desiccated thyroid which have a high content of iodine also contain a high percentage of "active" thyroxine which can bring about all of the physiological effects of thyroxine, can withstand hydrolysis with sulfuric acid and retain its activity, but cannot be boiled with sodium hydroxide and subsequently be isolated as thyroxine.

THE INFLUENCE OF OTHER COMPOUNDS ON THYROXINE

In addition to the *in vitro* studies on the oxidation of thyroxine, investigations have been carried out concerning variations in its physiological activity *in vivo*. These have clearly indicated variations in the response to thyroxine which were produced by the presence of other substances. The quantitative response of both epinephrine and thyroxine depends not only on the diet but also on the presence of other physiologically active compounds in the body. The administration of large amounts of glucose prevents the typical effect of epinephrine on the basal metabolic rate or blood pressure (Boothby [59]). Abderhalden and Wertheimer [5] have shown that the influence of thyroxine is greatly altered by the presence of ergotamine.

Abderhalden and Wertheimer [5] also showed that the basal metabolic rate, after the administration of thyroxine, was increased but little, and the duration of the effect was short, when the diet consisted largely of carbohydrates, but that the increase in the heat production was greater and the influence was of longer duration when the diet consisted largely of protein material. Antagonism in the action of drugs has been recognized for many years, but the reason for the altered physiological response has but rarely been expressed in terms of chemistry. The inactivation of histamine by formaldehyde is an example of inactivation of the essential chemical group in a compound which possesses marked physiological activity (A. I. Kendall [264, 265]).

It is highly significant to show that the velocity of oxidation in the animal organism is so dependent on the presence of certain definite groupings: further study of the influence of these groupings may indicate the specific chemical reactions which are controlled by thyroxine and epinephrine.

When the thyroid gland is completely atrophied or when it is removed from an experimental animal, death does not result, but the basal metabolic rate drops to a point approximately 40 per cent below normal. A smaller decrease in the basal metabolic rate has been noticed after the removal of the suprarenal glands. It would thus appear that the presence of thyroxine and epinephrine in the functioning cells of an animal organism produces an alteration in the velocity at which chemical reactions occur and there can now be but little doubt that the reactions which are affected deal directly with the consumption of oxygen and the production of carbon dioxide.

It cannot yet be definitely stated that thyroxine can exist in oxidized

and reduced forms, but there can be no doubt that the physiological activity of epinephrine is in large measure dependent on the presence of two hydroxyl groups in the benzene ring. This fact, taken together with the influence of hydroxyl derivatives in plant life, specifically chlorogenic acid (Oparin [373]), and the respiration chromogens of Palladin,[290] indicates the importance of hydroxyl compounds, which modify the velocity of oxidative processes. These substances have the ability to act as "hydrogen acceptors," and to bring about interaction of hydrogen donators and oxidizing substances, including molecular oxygen.

Oppenheimer reviewed this subject and pointed out that nothing occurs in these cases except that the hydrogen is taken up by the acceptor and then the leuco form of the dye turns it over to the oxygen and in this process the reaction is accelerated. He concluded by saying, "This is the fact, the explanation is not available." As a tentative hypothesis it seems highly probable that both thyroxine and epinephrine may be members of that group of substances which function in this manner and that the physiological activity of these compounds is at least in part due to their influence on the velocity of oxidative processes in the tissues of the animal organism. Whether or not this specific reaction occurs in all cells or whether the action is confined to the liver or other special organ has not been shown.

SUMMARY

No definite chemical reactions are known which are specifically influenced by thyroxine. It has been clearly shown that protein, fats and carbohydrate are all more rapidly oxidized after administration of thyroid material. The remarkable influence of hydroxyl benzene derivatives on oxidation in plants and the influence of epinephrine on *in vitro* oxidation suggests that thyroxine may exist in oxidized and reduced forms and that it may function as a direct catalyst in oxidative processes. Thyroxine is not oxidized by indigo carmine, methylene blue or dibromophenolindophenol at pH 7.4. Active thyroxine may be sensitive to oxidizing agents but it is not sufficiently stable to alkali and acid to withstand hydrolysis from the protein.

Chapter 21

The Influence of Thyroxine and Epinephrine on Biological Oxidations

The physiological activity of thyroxine and of epinephrine appears to depend in large part on their effect on processes of oxidation in the animal organism. The weight of material required suggests that they act as catalysts. Studies in respiratory metabolism and in oxidation *in vitro* have afforded an approach to this problem which has already resulted in significant progress.

The calorigenic action of epinephrine has been quantitatively demonstrated by Boothby and Sandiford.[66] Soskin confirmed this work but failed to secure calorigenic action of epinephrine in an eviscerated or hepatectomized dog. There was no effect on the usual decline in the oxygen intake, and injection of epinephrine did not show a significant influence on the respiratory quotient. Soskin concluded that the calorigenic action of epinephrine does not depend on direct stimulation of tissue cells. He considered the presence of the liver necessary for such an effect.

Aub, Bright and Uridil [30] have shown that the increased metabolism after the administration of thyroxine cannot be explained by muscular activity, muscular fibrillation or increased muscular tonus. The suprarenal glands were not essential to the maintenance of high metabolic rates induced by thyroxine. They concluded that thyroxine stimulated the resting cell to a higher level of activity.

Whether or not the sympathetic nervous system is directly involved in the functioning of thyroxine has not been definitely shown. Magnus-Levy [330] felt that the thyroid could act through the nerves directly or indirectly on the tissues of the body. Krogh [296] found that desiccated thyroid produced a much smaller increase in basal metabolic rate in a frog after all the nerves to the extremities were cut. She concluded that the augmentation of metabolism is caused by an increased muscular tonus through irritation of the autonomic nervous system. Abderhalden and Wertheimer [5] suggest that the action of thyroxine is through the

sympathetic nerve. The reason for this is based on the influence of ergotamine which inhibits the function of the sympathetic nerve. They have shown that after administration of ergotamine had brought about a decrease in temperature and a decrease in the metabolic rate there was a return to normal, but after normal conditions had been reached the administration of thyroxine was not followed by a physiological response; therefore they assumed that the sympathetic nerves had remained under the influence of ergotamine. When thyroxine did again affect the basal metabolic rate it was assumed that the nerves had come out from under the effects of the ergotamine.

IN VITRO OXIDATION

Definite evidence as to the influence of the thyroid on oxidation in body tissue, *in vitro,* has been obtained. The most significant result is that obtained by Rohrer,[423] who showed that the oxygen uptake of the tissues of a mouse was increased after the administration of thyroid material. The percentages of the increased oxygen uptake based on that of the normal mouse tissue are clearly shown in the following table:

TABLE 15. *Oxygen Uptake of 1 Gram Normal and Thyroid-Fed Tissue (Rohrer)*

	Cubic Millimeters of Oxygen	Per Cent Increase
Normal liver of mouse	639	22.6
Thyroid-fed	784	
Normal kidney of mouse	818	15.6
Thyroid-fed	941	
Normal muscle of mouse	791	23.9
Thyroid-fed	985	

These results show that there is an increased consumption of oxygen not only of the entire animal, but also of isolated organs following thyroid administration; however, the increase above the normal varies with different organs.

Significant changes, although more difficult of interpretation, have also been found by Neuschlosz, who showed that the velocity of reduction of dinitrobenzene was increased in both normal and cancer tissue by the addition of desiccated thyroid.

Ahlgren [17] also found that minute amounts of thyroxine accelerated the reduction of methylene blue by tissue. The muscle of thyroidectomized rabbits was found to have a lower rate of reduction of methylene blue than normal muscle. The addition of thyroxine increased the velocity of reduction of methylene blue to the normal figure, but it did

not increase the velocity of reduction of this dye in normal rabbit muscle.

Interpretation of these results in regard to the function of thyroxine in the animal organism is not clear. The evidence concerning the velocity of reduction of methylene blue shows quite clearly that the presence of thyroxine influences this reaction, but whether the influence so exerted is similar to the physiological functioning of thyroxine in the animal organism is not so clear. That tissue *in vitro* can utilize thyroxine more rapidly than it can be incorporated in the normal living protoplasm does not seem probable.

One significant difference between the conditions which exist in an evacuated Thunberg apparatus and that found in the functioning animal organism is the concentration of oxygen. The reduction of methylene blue is a measure of the velocity of oxidation only in the complete absence of oxygen. Whether the presence of oxygen would increase the influence of thyroxine on the activity of the tissues, or whether the apparent influence of thyroxine would disappear when oxygen was admitted to the tissues, are questions which must still be answered. Further development of this type of investigation will be awaited with interest.

It seems highly probable that the essential chemical reactions which are modified by the presence of thyroxine and epinephrine are closely related, but the mechanism by which these reactions are influenced by these two agents may be very different. This is well illustrated by the influence of these two substances on carbohydrate metabolism. Both thyroxine and epinephrine increase the mobilization of sugar, and there can be no doubt that this is through the conversion of glycogen in the liver into glucose. This phase of thyroid activity has been well presented in a recent article by Britton and Myers. They have shown that thyroidectomized cats are more sensitive to hypoglycemia after the administration of insulin, but this effect may be only transitory. Ablation of the suprarenal medulla after thyroidectomy resulted in an extreme and persistent reduction of the normal ability to withstand insulin.

Thyroxine has the opposite effect on carbohydrate storage to that of epinephrine: the liver may be completely depleted of glycogen through the action of thyroxine, but the ultimate effect of epinephrine on the liver is to increase its store of glycogen. There may be a transfer of muscle glycogen to the liver after an injection of epinephrine. This was first shown by Pollak,[406] and his results have been confirmed by Cori and Cori.[109]

The physiological effect of thyroxine differs from that of epinephrine in the influence on nitrogen metabolism. There is no increased excretion of nitrogen following the injèction of epinephrine; however, the basal metabolic rate cannot be changed by the administration of thyroxine without a simultaneous increase in nitrogen elimination. The effect of thyroxine on nitrogen metabolism is not due to the fact that proteins are metabolized because of lack of carbohydrates and fats, but the influence on nitrogen output appears to be due to an alteration in the equilibrium which exists within the cells. What proteins are influenced is unknown, except that sulfur metabolism is not increased.

Boothby, Sandiford, Sandiford and Slosse [69] have suggested that there is a relationship between the deposit protein and the basal metabolic rate. Some recent results reported by Berglund and Medes show that in certain cases of nephrosis the quantity of protein excreted in the urine is markedly increased by thyroid administration. The whole extra nitrogen output in the urine after administration of thyroid was in the form of protein. This confirms the hypothesis suggested by Boothby and his coworkers that other factors being left constant the amount of deposit protein depends on the thyroxine concentration in the tissues and that there is only a temporary mobilization of protein material following the administration of thyroxine.

An increased output of nitrogen, however, and an increased basal metabolic rate has been observed after the injection of other substances, notably inorganic iodides (Hesse) and proteinogenous amines (Abelin [10]). The effects of thyroxine, iodides and amines on the excretion of nitrogen, protein metabolism and basal metabolism, cannot be expressed in terms of chemistry. It is hoped that more work will be done on this phase of thyroid activity for it is of fundamental importance to our knowledge of the physiology of the cell.

MOBILIZATION OF METABOLITES

The calorigenic action of thyroxine and epinephrine may be explained by the specific action of these substances on the chemical reactions which are associated with processes of oxidation. This, however, is not the only possible explanation. Thyroxine and epinephrine may not directly affect the intensity of or capacity for oxidation in the animal organism: they may act as regulators for the mobilization of metabolites. The reaction mechanism which is concerned with the complete oxidation of proteins, fats and carbohydrates may function entirely independently of thyroxine or epinephrine. These two substances, however, by their

effect on the delicately balanced mechanism which controls the mobilization of metabolites may control the velocity of mobilization of the material available for combustion and in this way determine the level of basal metabolism.

Administration of epinephrine results in an almost immediate increase in the respiratory quotient which indicates the combustion of carbohydrates. Analysis of the blood shows conclusively that epinephrine exerts a dominating influence on the mobilization of carbohydrates. These results are in keeping with the hypothesis that epinephrine

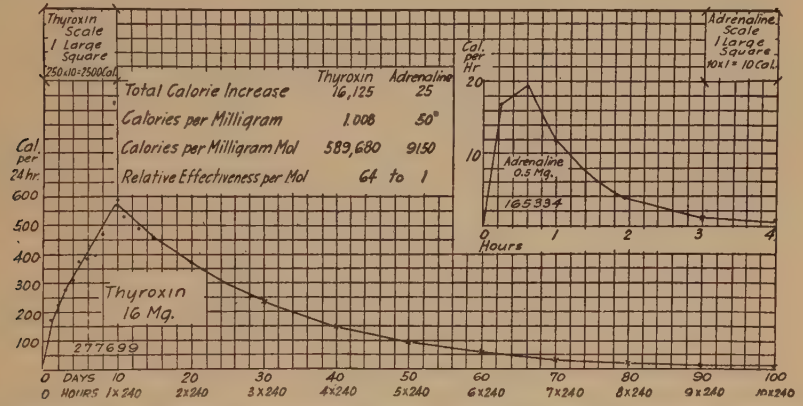


FIG. 39.—Curve showing response in basal metabolic rate to epinephrine expressed in hours and to thyroxine expressed in days. (Boothby and Sandiford. *J. Biol. Chem.*, 1924, lix, xl.)

modifies the rate of mobilization rather than the actual oxidation of carbohydrate.

The administration of thyroxine does not increase the respiratory quotient, and there may be a decrease in this index for the type of combustion. The blood sugar may not be elevated, but the velocity of oxidation of proteins, fats and carbohydrates is increased. This observation is in keeping with the hypothesis that thyroxine in addition to a possible influence on the mobilization of metabolites may specifically affect the reaction mechanism which is involved in biological oxidations.

The greatest difference between the action of thyroxine and of epinephrine is found in the time during which these agents continue to function. Epinephrine ceases to have any influence on oxidative processes sixty minutes after it has been injected in amount sufficient to produce a physiological, but not pathological effect. The effect of a single injection of thyroxine may be continued for four or five weeks.

(Fig. 39.) Thyroxine appears to furnish a pace-maker for the velocity of chemical reactions which occur intracellularly, and it is possible that the thyroxine molecule produces a specific sphere of influence which alters the metabolic activities of the individual cell and therefore of the tissues. Such a concept explains in part the delayed action of this substance: thyroxine can bring about its characteristic effect on the production of energy only after it has assumed its essential position in the protoplasmic structure. After it has been incorporated into the functioning protoplasm it continues to affect the velocity of reaction for a period of time which is not equaled by any other known substance. These observations may furthermore furnish an explanation of the discrepancies which have been observed concerning the acute and chronic effect brought about by thyroid medication. It is interesting to note that the latent period after the administration of desiccated thyroid is just as long as the delay following an injection of thyroxine: the basal metabolic rate requires the same long interval, from six to ten days, before its maximal response is reached.

CAN THE THYROID ELABORATE AN ABNORMAL PRODUCT?

Although a latent period is associated with the activity of thyroxine, this fact does not exclude the possibility that some product may be elaborated in the thyroid gland, which may or may not be a derivative of thyroxine, and which may produce an immediate but transitory effect on basal metabolism. Plummer [401] has suggested that in its action this hypothetical compound resembles epinephrine much more closely than it does thyroxine. A study of certain cases of exophthalmic goiter strongly suggests that the thyroid gland under some conditions may elaborate such a compound.

An unusual clinical condition has been reported by Haines. The patient was a woman aged twenty-eight who had been operated on for exophthalmic goiter. Sufficient of the gland had been removed to relieve this condition, but bilateral exophthalmos persisted, and a few months after the operation she again became nervous and had palpitation and other symptoms of exophthalmic goiter. A compound solution of iodine was given, and the nervousness and exophthalmos were relieved; however, continuation of the iodine brought on a condition which was typically that of myxedema. The basal metabolic rate was 30 per cent below normal. After iodine had been administered for several months it was stopped and the basal metabolic rate rose, after sixteen days, to 14 per cent below normal. The patient then complained of nervous-

ness, the edema of the eyelids was diminished, and a stare characteristic of exophthalmic goiter was present. A compound solution of iodine was again given and after eighteen days the basal metabolic rate was again 25 per cent below normal. Evidence of myxedema and loss of the stare and nervous manifestations followed the administration of iodine. It was evident that iodine was required to control the symptoms of exophthalmic goiter but the low basal metabolic rate indicated the need of thyroxine. In addition to the iodine desiccated thyroid was given. The basal metabolic rate rose to practically normal. Evidence of myxedema disappeared and there was considerable improvement in the patient's symptoms. These results indicated that there was an absolute deficiency of the thyroid secretion and what secretion was present was partially of the character of that produced in exophthalmic goiter. He pointed out that several cases have been seen in The Mayo Clinic presenting the syndrome of exophthalmic goiter and with normal basal metabolic rates dropping to 15 per cent below normal after administration of iodine. "This case, however, is the only one observed in the Clinic in which the basal metabolic rate was depressed because of the administration of iodine to so low a level as to be consistent and coincident with the characteristic frank manifestations of myxedema."

In this case it is possible that thyroxine is not the only physiologically active compound which passed into the blood stream or lymph from the thyroid gland. The exophthalmos and nervous manifestations cannot be produced either by an excess or absence of thyroxine. On the other hand, the decrease in the basal metabolism, the relief of the nervous symptoms and of the exophthalmos following the administration of iodine suggest that an abnormal product which was produced could not be elaborated and secreted by the thyroid gland when sufficient iodine was administered.

This type of evidence, although it lacks the proof furnished by the isolation of definite chemical substances, clearly indicates the possibility of the elaboration of substances in the thyroid gland or other parts of the body which can increase the basal metabolism and bring about a physiological response which is not identical with that produced by thyroxine. It is altogether possible that the thyroid gland may, under conditions such as exist in exophthalmic goiter, elaborate a substance which radically alters the physiological response to thyroxine. This effect could be anticipated from the marked influence of certain chemical groupings on the reactivity of epinephrine and related compounds. Whether or not the thyroid gland, when in the status known as exoph-

thalmic goiter, produces a second substance which modifies the action of thyroxine, or whether an abnormal product is directly responsible for the increase in the basal metabolism and for the effect on the nervous system, cannot be stated. The influence of Lugol's solution on the thyroid gland as it exists in exophthalmic goiter is suggestive of a modification in the chemical properties of the secretion elaborated by the gland with a return to a more normal status and function.

THYROXINE IN THE BLOOD AND TISSUES

In regard to the chemical nature of the vehicle by which thyroxine is carried the experimental results have been uniform. They indicate that active thyroxine leaves the thyroid gland in combination with the protein molecule.

Zavadovsky and coworkers [534, 535, 536, 537, 539] have carried out an extended investigation on the determination of thyroid material in the blood and tissues of animals after the administration of desiccated thyroid. For a criterion they used implantation of the material in the axolotl and noted the velocity of metamorphosis which was produced. Romeis, Huxley and others have shown that the velocity of metamorphosis is proportional to the iodine content of the thyroid material. The results of Zavadovsky showed that thyroxine was not transported in the corpuscles or in the fibrinogen: its presence was demonstrated in the serum of the animal to which it had been administered. The blood of chickens after a single administration of thyroid material brought about striking changes in the axolotl. This property was found to last in the blood from nine to ten days. The liver and kidney brought about the same effect but they were more active. Thyroid material was shown to be present in the blood, liver and kidney of a guinea-pig. Evidence of similar nature is furnished by the work of Hektoen, Carlson and Schulhof [208] on the presence of thyreoglobulin in the blood and lymph.

There is no evidence that any protein-free extract of the thyroid gland brings about the most characteristic physiological response of thyroxine—an increase in basal metabolism. This statement was first shown to be true by Magnus-Levy with thyreo-antitoxin. The results of Eiger and Abelin and others of the Asher school on almost protein-free extracts of the thyroid gland do not prove the presence of an iodine-free active agent. Romeis [425] has demonstrated the extraordinary physiological activity of thyroxine in regard to the metamorphosis of the tadpole and he suggests that thyroxine does not exist uncombined in the thyroid secretion but is attached to another substance. Since the

"almost protein-free" extract contained small amounts of iodine its entire activity can be explained by the presence of active thyroxine. The fact that every symptom associated with hypothyroidism is relieved by thyroxine alone is incontrovertible evidence that no other agent is required.

If it is assumed that all products which are elaborated by the thyroid gland and which possess physiological activity are intimately bound within the protein molecule, it at once becomes evident that chemical investigation of the problem of the differentiation between normal and abnormal secretions can be approached through but a narrow gateway. No substance with physiological activity, other than thyroxine, is sufficiently stable to withstand the drastic hydrolysis required to separate it from the protein; therefore, any investigation of the chemical difference between normal and abnormal secretions must be confined to a study of the extracts of the thyroid in the form of proteins. This very fact, however, is significant and is in keeping with the hypothesis concerning the nature of the active constituent of the thyroid, and the processes affected by the thyroid gland. These processes of oxidation are of a fundamental nature which involve an equilibrium not easily altered.

THYROXINE AND EPINEPHRINE

Rapid fluctuations in the production of heat and energy in the body are apparently not brought about by rapid fluctuations of the thyroxine content of the tissues. An increase in the actual concentration of the thyroxine, which functions within the cells of the animal organism, may require a period of time measured in days. It is obvious that such a mechanism is too fixed and stable to furnish the necessary alterations in the output of heat and energy to meet the constantly changing demands of the animal organism.

If the physiological effect of the thyroid secretion were exerted over a long period of time then it could be predicted that the molecular weight of the active agent would be high, that it would be securely attached to a still more bulky vehicle for transportation and that it would ultimately be incorporated in the functioning protoplasm in a manner which would insure its maintenance in an available form over a maximal period of time. These predictions are fulfilled by all that is known in regard to the essential nature of the thyroid secretion.

The chemical nature of epinephrine is diametrically opposed to these findings. The molecular weight is low, it is easily diffusible, it is not attached to protein, it is poured directly into the blood stream from

which it is removed by the tissues; there is no delay in the manifestation of its effect, its maximal time of activity is but a few minutes, but its physiological effect during this interval is marked. Thyroxine furnishes a sustained driving force which maintains the basal level of processes of oxidation. Epinephrine has recently been shown to be essential for the normal response of the animal organism when placed under conditions which require a rapid increase in heat production (Britton) or a rapid increase in carbohydrate metabolism (Britton, Geiling and Calvery).

The complete elucidation of the chemical reactions which are so profoundly affected by epinephrine and by thyroxine and the relation of the active groups to oxidative processes in the body are among the most interesting and important problems which invite the attention of the chemist. The chemical investigation of thyroxine has been of more than passing interest because of the knowledge that one of the most significant secrets of biology is bound within its molecule. This single substance can transform flesh and blood from a mass of protoplasm into a rational being.

SUMMARY

Thyroxine can affect the basal metabolic rate in the absence of the suprarenal gland: the sympathetic nervous system influences the physiological effect of thyroxine. *In vitro* oxidation is more rapid in tissue removed from an animal which has previously received thyroid material. Although thyroxine may act specifically on oxidative processes in the cells, it may also have an important influence on the mobilization of metabolites.

A study of the clinical conditions associated with exophthalmic goiter has shown that after removal of the thyroid a condition resembling this disease may exist with a normal basal metabolic rate. Administration of iodides causes a decrease in the nervous symptoms and a marked drop in the basal metabolism; this may be so great that a condition of myxedema is produced. Thyroxine administered in this condition will bring about a normal basal metabolism with absence of nervousness and other symptoms of exophthalmic goiter. In exophthalmic goiter a dysthyroidism may exist and an abnormal product somewhat similar to epinephrine in its physiological effect may be elaborated by the thyroid.

Rapid fluctuations in the production of heat and energy are not produced by rapid fluctuations of the thyroxine content of the tissues. Thyroxine produces a long-continued acceleration of processes of oxidation. Epinephrine produces a rapid increase in heat and energy production which lasts but a few minutes.

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